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Molecular Properties of Carboxymethyl Chitosan and Its Complexes with Curcumin and Nicotinamide in Drug Delivery Applications: Molecular Docking and Molecular Dynamic Study

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Abstract

Carboxymethyl chitosan (CMCs) is a chitosan-derived compound usually used as a carrier matrix in drug delivery systems. There are three types of CMCs based on the location of carboxyl group substitution: N-CMCs, O-CMCs, and N,O-CMCs. The ability of CMCs as a carrier is related to the ability of CMCs to interact with drug molecules. In this work, curcumin and nicotinamide were used as drug models. The ability of CMCs to interact with drug models can be observed by the amount of interaction energy generated when CMCs interact with curcumin and nicotinamide. The purpose of this study is to determine the interaction energy generated when CMCs interact with curcumin and nicotinamide using molecular docking and molecular dynamic methods. The results showed the interaction energy between O-CMCs, N-CMCs, and N,O-CMCs (2 and 3 monomers) with curcumin and nicotinamide, respectively, ranged from -17.08 to -13.37 and -12.05 to -11.00 Kj/mol. Conformational changes in molecular dynamic simulations affect bond-free energy, RMSD, and potential energy complex values.

1. Introduction

Carboxymethyl chitosan (CMCs) or β-(1,4)-D-glucosamine is a chitosan-derived compound obtained from the carboxymethylation process on chitosan. Caprifico *et al.* [1] in their research explained that chitosan could be used to help drug delivery through the blood-brain barrier (BBB). However, one way to improve chitosan's function is to modify the functional groups in chitosan. One of the modifications is utilizing CMCs. Carboxymethylation can be performed on both the hydroxy and amino groups of chitosan, resulting in the production of three types of carboxymethyl chitosans (CMCs): N-CMCs, O-CMCs, and N,O-CMCs. CMCs exhibit excellent solubility in water and acid, along with biodegradability, biocompatibility, low toxicity, and high mechanical stability, making them suitable for utilization in drug delivery systems. Moreover, CMCs can serve as carriers for various model drug molecules [2].

The properties of CMCs as carriers in drug delivery systems can be reviewed by their ability to interact with other molecules. In this study, curcumin and nicotinamide are used as drug models that would be attached to CMCs. Curcumin and nicotinamide have simple molecular structures and are widely used in the medical field. Utilizing CMCs as carriers for curcumin and nicotinamide compounds in drug delivery systems can demonstrate a controlled and gradual release of these compounds over time. The ability of CMCs to release curcumin and nicotinamide gradually is thought to be related to the interaction energy between CMCs with

curcumin and nicotinamide. The amount of interaction energy is difficult to determine through experiments but can be done by computational modeling of molecular mechanics.

Siahaan *et al.* [3] conducted an in vitro study demonstrating the efficacy of nicotinamide encapsulation by CMCs, achieving an encapsulation efficiency ranging from 63.95% to 69.71%. Furthermore, employing a molecular docking method, the interaction energy between CMCs and the nicotinamide complex was calculated to be -3.49 kcal/mol. A study by Jannah *et al.* [4] employed a computational study using molecular mechanics to investigate the interaction between CMCs and vitamin C. It revealed that the strength of the energy in the interaction complex significantly influenced the controlled release mechanism.

Hence, this research aims to explore the effects of altering the position of carboxyl group substitutions in CMCs, alongside variations in CMCs segments (dimer and trimer) modeled as matrix substances carrying curcumin and nicotinamide molecules. The interaction between CMCs with curcumin and nicotinamide produces CMCs···curcumin and CMCs···nicotinamide complexes. The resulting interaction energy can be calculated using YASARA software (version 23.12.24). In the computational calculation, geometry optimization was carried out on CMCs, curcumin, and nicotinamide molecules to obtain the most stable geometry structure. The results of the structure of the CMCs molecular geometry optimization interacted with curcumin and nicotinamide. The molecular mechanics method with the docking procedure can explain the interactions that occurred between CMC molecules with curcumin and nicotinamide in forming complexes. In addition, molecular mechanics can estimate conformational changes, ligand binding, and receptor folding over time.

2. Experimental

2.1. Materials

The tools used in this research were a set of computers with YASARA software. The initial geometry of all molecules was made using Avogadro. The calculation input used was the molecular structures (3D) of O-CMCs, N-CMCs, N,O-CMCs, curcumin, and nicotinamide. Each molecule's molecular weight is 368.4 g/mol for curcumin, 122.127 g/mol for nicotinamide, and 559.5 g/mol for CMCs.

2.2. Optimization of Receptor and Ligand Molecular Geometries

The initial step before geometry optimization was the preparation of 3D molecular structures of CMCs (O- CMCs, N-CMCs, N,O-CMCs) with variations in dimer and trimer, curcumin and nicotinamide with Avogadro software. The 3D structure of the molecule went through a minimization stage using YASARA with the em_run.mcr command. The optimized structure was then ready for molecular dynamic simulation using YASARA. The purpose of geometry optimization was to obtain the most stable conformation of a molecule. The parameters used in molecular dynamic simulations were a time duration of 50 ns (nanosecond) with a timestep of 2.5 fs (femtosecond) and trajectory results calculated every 50 ps (picosecond). The solvent system parameter used was TIP3P in a solution pH of 7.4, and the density used was 0.997 g/L, NaCl buffer solution with a concentration of 0.9% [5].

2.3. Molecular Docking Simulation of CMCs, Curcumin, and Nicotinamide Molecules

The molecular docking was done by treating CMCs as receptors and curcumin and nicotinamide as ligands. The set of distinguished input files was prepared from each optimized molecule structure. The grid box used was 10×10×10 Å. The data generated by this calculation were the binding energy, the interaction site, and the interaction distance of receptor-ligand docking.

2.4. Molecular Dynamic Simulation on Complex Molecules of CMCs···Curcumin and CMC···Nicotinamide

A molecular dynamic simulation was performed after CMCs, and curcumin and nicotinamide molecules were linked with molecular docking simulation. In molecular dynamic simulation, there are fixed parameters: water solvent, 0.9% NaCl buffer solution, 50 ns of speed duration time, pH solution of 7.4, and density of 0.997 g/L [5]. The molecular dynamic simulation process consisted of three main stages: equilibration, production, and calculation of Molecular Mechanics Poisson-Boltzman Surface Area (MM/PBSA) to assess the free energy of bonds within the complex. The analysis of the simulation yielded several key parameters, including total potential energy, changes in receptor-ligand interactions throughout the simulation, Root Mean Square Deviation (RMSD), and bond-free energy (ΔG) [2].

Table 1. The result of optimization energy of ligand and receptor using YASARA

Receptor and ligand	Before optimization (kJ/mol)	After optimization (kJ/mol)
Curcumin	-110.09	-85917.27
Nicotinamide	-364.29	-70945.84
O-CMCs-2	-636.24	-69774.44
N-CMCs-2	-760.16	-69581.51
N,O-CMCs-2	-719.90	-69570.87
O-CMCs-3	-936.36	-69074.69
N-CMCs-3	-1293.63	-68760.75
N,O-CMCs-3	-1398.34	-68894.98

Table 2. Changes in molecular length before and after optimization

Receptor and ligand	Molecular length (nm)	
	Before optimization	After optimization
Curcumin	1.70	1.76
Nicotinamide	0.70	0.71
O-CMCs-2	1.05	1.01
N-CMCs-2	1.14	1.14
N,O-CMCs-2	1.08	1.03
O-CMCs-3	1.58	1.37
N-CMCs-3	1.48	1.73
N,O-CMCs-3	1.43	1.33

3. Results and Discussion

3.1. Preparation and Optimization of Receptor and Ligand Geometries

The initial structures of CMCs, curcumin, and nicotinamide molecules were described in 3D and optimized using data-based methods in Avogadro software. Subsequently, the optimized structures were re-optimized using YASARA software. Geometry optimization via molecular dynamic simulation aids in identifying the most stable conformation of a molecule, typically indicated by a negative value of optimization energy [6]. Table 1 compares the results of optimization energy before and after optimization with molecular dynamics, which shows that the molecular structure becomes more stable after optimization with molecular dynamics, which is indicated by changes in optimization energy to be more negative. In addition to changes in optimization energy, the conformation of the molecule also changes, which can be shown in Figure 1.

Figure 1 shows the most stable conformation of the receptor and ligand molecules characterized by changes in molecular length before and after optimization using YASARA. Changes in molecular length can occur because atoms in a molecule experience attraction or repulsion. Changes in molecular length before and after geometry optimization are shown in Table 2. These changes can be attributed to variations in bond lengths, molecular angles, and dihedral angles within the molecule.

3.2. Intermolecular Interaction of CMCs···Curcumin and CMCs···Nicotinamide Using Molecular Docking Method

Intermolecular interaction between CMCs and both nicotinamide and curcumin was analyzed using molecular docking simulation using YASARA software. The molecular docking method aims to interact receptor molecules with ligands and predict the amount of interaction energy produced [7]. The type of interaction formed in the complex is a hydrogen bond interaction. Hydrogen bond interactions play a major role in compound stability [8]. Lower bond energies represent better receptor/protein-ligand binding affinity than higher energies [9]. The following visualizations of CMCs···curcumin and CMCs···nicotinamide complexes with variations in the number of monomers and the position of the carboxyl group substitution (-CH_2COOH) on the CMCs molecule are shown in Figures 2 and 3.

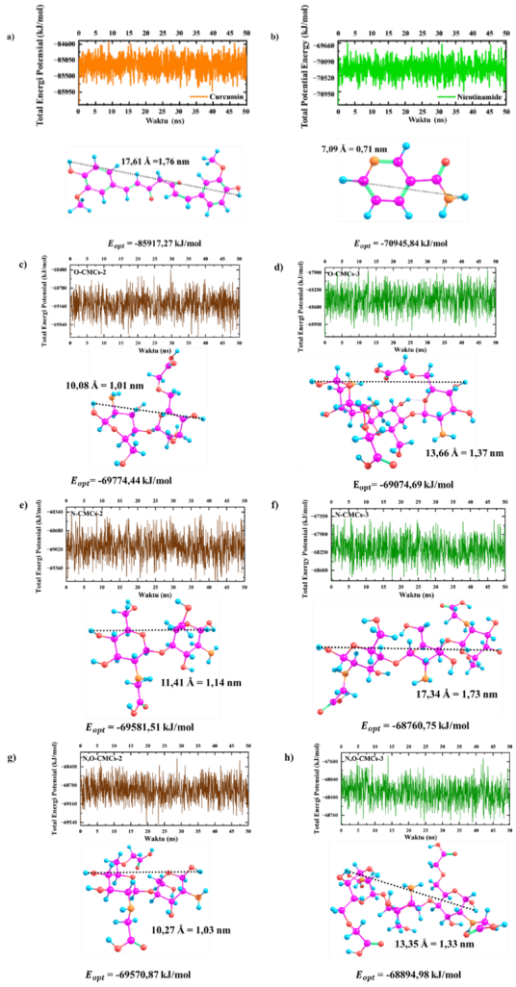

Figure 1. Conformation of stable structure of a) curcumin, b) nicotinamide, c) O-CMCs-2, d) O- CMCs- 3, e) N-CMCs-2, f) N-CMCs-3, g) N,O-CMCs-2, and h) N,O-CMCs-3

Figure 2 shows the highest and lowest interaction energies of the N-CMCs-2···curcumin and N-CMCs-3···curcumin complexes are -13.37 and -17.08 kJ/mol, respectively. The complex with the strongest possible interaction belongs to the N-CMCs-3···curcumin complex, which is also classified as a strong interaction because the interaction energy is <-16.8 kJ/mol [11]. This interaction can occur because CMCs have active sites on their chains that allow them to interact with curcumin. CMCs and curcumin interact, resulting in an interaction distance of 2.07-2.66 Å, which places the interaction into the weak group.

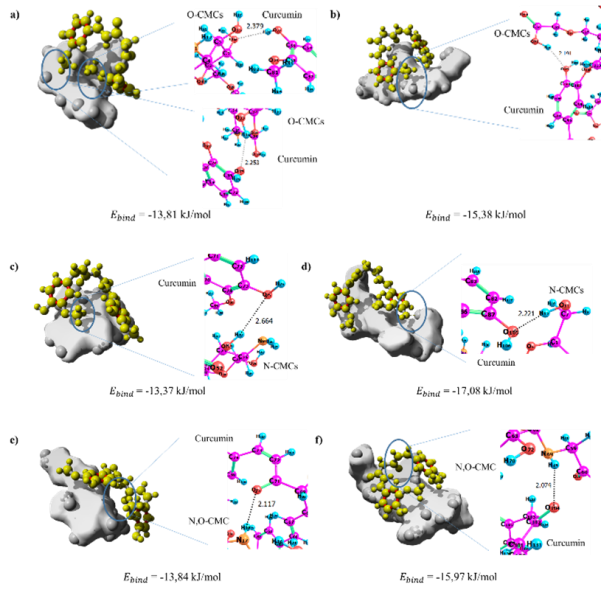

Figure 2. The interaction of CMCs with curcumin results in a complex of a) O-CMCs-2···curcumin, b) O-CMCs-3···curcumin, c) N-CMCs-2···curcumin, d) N-CMCs-3···curcumin, e) N,O-CMCs-2···curcumin, f) N,O-CMCs-3···curcumin

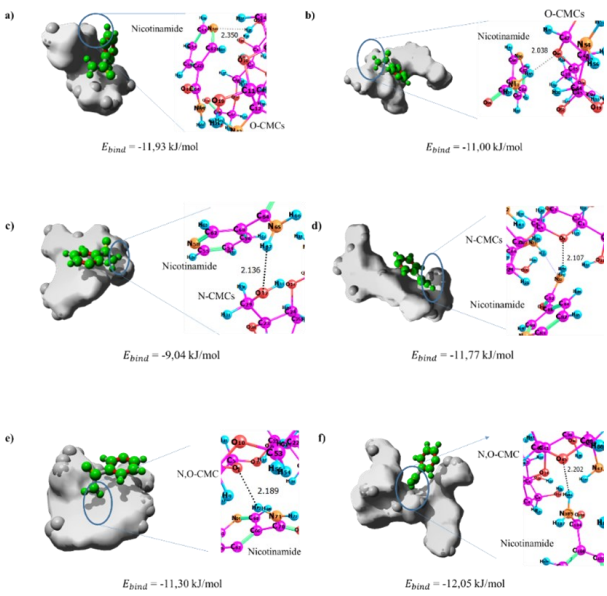

Figure 3. The interaction of CMCs with nicotinamide results in a complex of a) O-CMCs-2···nicotinamide, b) O-CMCs-3···nicotinamide, c) N-CMCs-2···nicotinamide, d) N-CMCs-3···nicotinamide, e) N,O-CMCs-2···nicotinamide, f) N,O-CMCs-3···nicotinamide

Figure 3 shows that the highest and lowest interaction energies are owned by the N-CMCs-2···nicotinamide and N,O-CMCs-3···nicotinamide complexes with values of -9.04 and -12.05 kJ/mol, respectively. The complex with the strongest possibility of interaction belongs to the N,O-CMCs-3···nicotinamide complex. The CMCs···nicotinamide complex is classified as a weak interaction because the interaction energy is >- 16.8 kJ/mol [10]. O-CMCs and curcumin interact, resulting in an interaction distance of 2.04–2.36 Å, which places the interaction into the weak group [11].

3.3. Analysis of Molecular Dynamic Simulation Results

Molecular dynamic is used to analyze the stability of the interactions of the CMCs···curcumin and CMCs···nicotinamide complex interactions as well as the discontinuation over time of variations in the number of CMCs monomers and the position of carboxyl group substitution [12]. The results of the analysis of CMCs···curcumin and CMCs···nicotinamide complexes using molecular dynamic simulations change in complex conformation, bond-free energy, root mean square deviation (RMSD), and potential energy.

3.4. Bond-free Energy Analysis of CMCs···Curcumin and CMCs···Nicotinamide Complexes

Bond-free energy is used to predict the bond strength between the receptor and the ligand [13]. As the bond-free energy increases, the interaction between the ligand and receptor weakens. Conversely, a low bond-free energy suggests a strong interaction between them. An increase in the energy value of the bond molecule signifies the release of the bond between the receptor and the ligand. To calculate the binding free energy of the ligand-receptor complex, the procedure involves capturing 1000 snapshots of the trajectories from molecular docking simulations results of CMCs···curcumin and CMCs···nicotinamide for 50 ns.

Figures 4 and 5 show the conformational changes of O-CMC···curcumin and O-CMC···nicotinamide complexes during the simulation, ranging from 0 to 50 ns. During molecular dynamics simulations, there are large increases and decreases in potential energy, and complex conformational changes occur during the simulation period. The initial step of molecular dynamic simulation on the complex is solvation with a solvent system, followed by adding counter ions (Na^+ and Cl^-) [4]. Next, the complex goes through a minimization stage and an equilibration stage.

Based on Figure 5, it can be seen that the bond is released in the complex between the ligand and the receptor. Conformational changes can be influenced by the amount of bond-free energy generated during molecular dynamic simulations. The increase and decrease in the magnitude of the bond free energy causes movement in the complex, resulting in conformation changes of the complex from time to time until the dynamic molecular simulation ends. The addition of solvent is crucial in molecular dynamics since it will affect the minimization energy. The solvent used in this work is based on the solvent used in the experimental study [5]. Regarding interaction energy, the solvent will stabilize the interaction between receptors and ligands.

3.5. RMSD Analysis of CMCs···Curcumin and CMCs···Nicotinamide Complexes

RMSD analysis aims to determine the description of how far the state of the receptor-ligand complex changes over time and determine the stability of the receptor-ligand complex structure [14]. The average RMSD of CMCs···curcumin and CMCs···nicotinamide complexes with variations in the number of monomers and the position of carboxyl group substitution (-CH_2COOH) on CMCs (O-CMCs, N-CMCs, N,O-CMCs), and their standard deviations are shown in Figure 6.

Table 3. RMSD mean and standard deviation of CMCs···curcumin complex

RMSD	
Complex	Mean + standard deviation (Å)
O-CMCs-2···Curcumin	7.73 ± 2.83
O-CMCs-3···Curcumin	7.22 ± 2.61
N-CMCs-2···Curcumin	7.55 ± 2.99
N-CMCs-3···Curcumin	7.04 ± 2.35
N,O-CMCs-2···Curcumin	7.99 ± 2.72
N,O-CMCs-3···Curcumin	6.43 ± 2.47

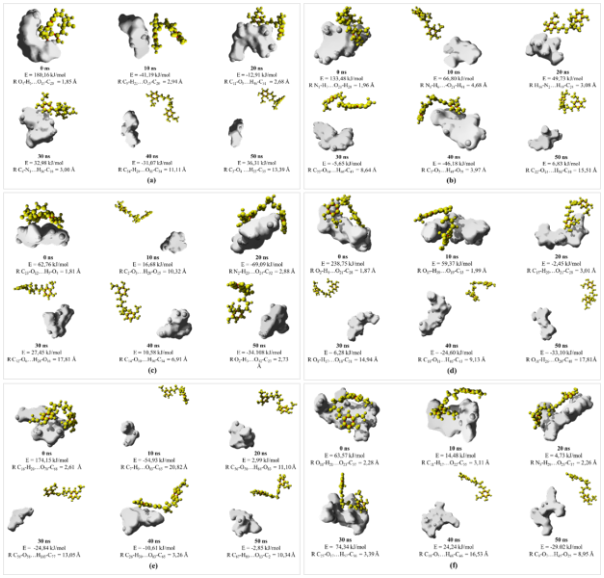

Figure 4. Controlled release mechanism of curcumin using molecular dynamic of (a) O-CMC-3···Curcumin, (b) O-CMC-3···Curcumin, (c) N-CMC-2···Curcumin, (d) N-CMC-3···Curcumin, (f) N,O-CMCs-2···Curcumin, (g) N,O-CMCs-3···Curcumin

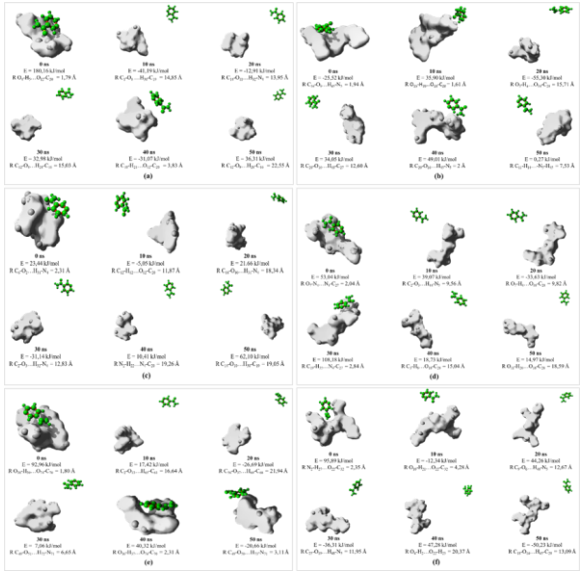

Figure 5. Controlled release mechanism of nicotinamide with molecular dynamic (a) O-CMC-2···Nicotinamide, (b) O-CMC-3···Nicotinamide, (c) N-CMC-2···Nicotinamide, (d) N-CMC-3···Nicotinamide, (e) N,O-CMCs-2···Nicotinamide, (f) N,O-CMCs-3···Nicotinamide

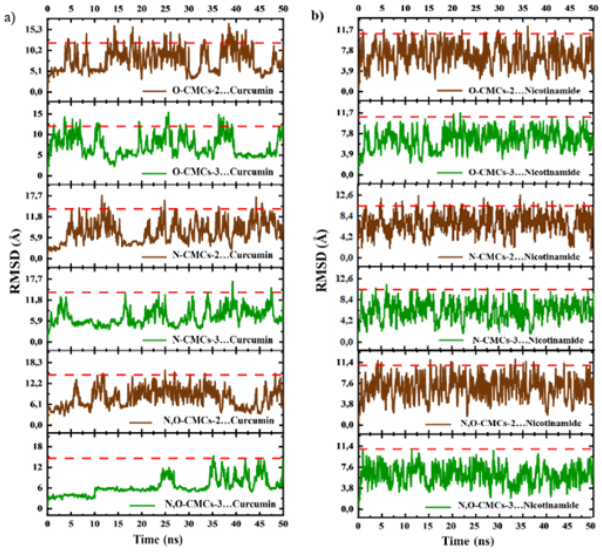

Figure 6. Fluctuations of RMSD of (a) CMCs···curcumin complex and (b) CMCs···nicotinamide complex

In Figure 6, the stability of the graph is obtained from the RMSD value of the CMCs···curcumin and CMCs···nicotinamide complexes with variations in the number of CMC monomers and substitution position of the carboxyl group. The results show that each monomer in CMC has different stability. Complex stability produces constant fluctuation graphs [15]. Differences in RMSD values over time indicate conformational deviations in the receptor structure due to interactions with ligands. The lower the average RMSD value, the more stable the ligand-receptor complex [16]. RMSD is calculated based on the difference in receptor backbone to identify conformational changes in the receptor because the receptor backbone can represent the conformation of the receptor [17].

Tables 3 and 4 show that the N,O-CMCs-3···curcumin and N,O-CMCs-3···nicotinamide complexes have the lowest average RMSD with values of 6.43 ± 2.47 and 6.43 ± 2.47 Å, respectively. According to the graph of the increase in RMSD values, it can be seen that the number of monomers in CMCs and the substitution position of the carboxyl group can affect the stability of the complex conformation over time for 50 ns. Changes in the stability of complexes can be caused by conformational changes due to complex motions.

Table 4. RMSD mean and standard deviation of CMCs···nicotinamide complex

RMSD	
Complex	Mean + standard deviation (Å)
O-CMCs-2···Nicotinamide	6.86 ± 2.19
O-CMCs-3···Nicotinamide	6.64 ± 1.86
N-CMCs-2···Nicotinamide	7.08 ± 2.04
N-CMCs-3···Nicotinamide	6.32 ± 1.92
N,O-CMCs-2···Nicotinamide	6.83 ± 2.08
N,O-CMCs-3···Nicotinamide	6.18 ± 1.68

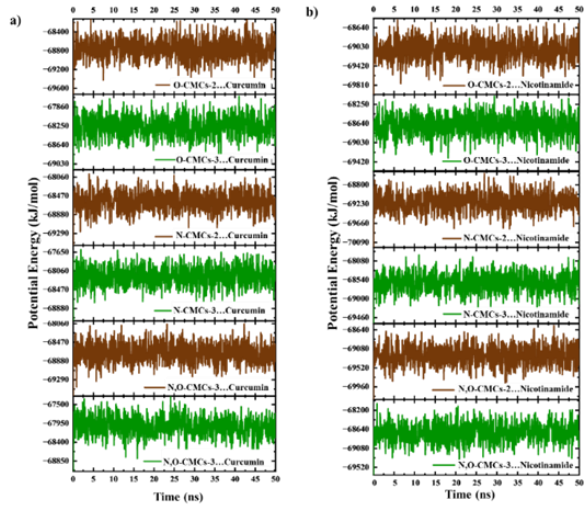

Figure 7. Fluctuation of the total potential energy of a) CMCs···curcumin and b) CMCs···nicotinamide complexes

3.6. Potential Energy Analysis of CMCs···Curcumin and CMCs···Nicotinamide Complexes

Potential energy analysis aims to determine how much energy the receptor and ligand need to interact to form a complex [18]. During molecular dynamic simulations, the complex of CMCs···curcumin and CMCs···nicotinamide undergoes a conformational change, which can then be used to analyze the stability of the complex. A high potential energy value indicates the opening of a complex structure [19]. Overall, the CMCs···curcumin and CMCs···nicotinamide complexes have stable potential energies from 0.1–50 ns, which are characterized by stable fluctuations during the simulation.

Figure 7 is the graph of the total potential energy of the CMCs···curcumin and CMCs···nicotinamide complexes, which can explain the folding and unfolding case. According to Figure 7, fluctuations in potential energy suggest both the unfolding and folding of bonds within the complex. The graph in brown reflects the highest energy values, while the green one shows the lowest values for the various complex conformations. Molecular dynamic simulations yield numerous conformations, each associated with distinct potential energy levels. These variations stem from the interplay of attraction and repulsion interactions within the complex.

4. Conclusion

A molecular dynamics study utilizing YASARA computational software was conducted on CMCs···curcumin and CMCs···nicotinamide complexes, exploring variations in dimer and trimer CMCs segments. The results suggest that the binding energy of O-CMC···curcumin and O-CMC···nicotinamide complexes could influence the controlled release mechanism of curcumin and nicotinamide. Notably, the complexes with the best possibility of interaction were N-CMCs-3···curcumin and N,O-CMCs-3···nicotinamide with interaction energies of -17.08 kJ/mol and -12.05 kJ/mol, respectively. Throughout the molecular dynamic simulations, both CMCs···curcumin and CMCs···nicotinamide complexes underwent conformational changes, thereby affecting parameters such as bond-free energy, RMSD, and potential energy.

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