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Original Article

EXPLORING BINDING AFFINITIES OF ACETOACETATE IN ACRYLAMIDE-BASED POLYMERS (PAM) FOR MOLECULARLY IMPRINTED POLYMERS (MIPS): A MOLECULAR DOCKING AND MOLECULAR DYNAMICS STUDY

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ABSTRACT

Objective: Molecularly Imprinted Polymers (MIPs) have garnered significant attention as promising materials for the selective recognition of target molecules. Acetoacetate is crucial in diabetes management, especially in Type 1 diabetes and diabetic ketoacidosis (DKA), and monitoring its levels is essential for detecting potential complications. In DKA, there is a lack of insulin resistance, leading to increased production of ketone bodies, including acetoacetate. MIPs, synthetic polymers, selectively bind to target molecules like acetoacetate due to unique three-dimensional structures, which can be quantitatively measured using molecular docking and molecular dynamics simulations. The research objectives were to assess the stability of acetoacetate-MIP complexes and their impact on polyacrylamide-based polymer (PAM) using molecular docking and molecular dynamics, examining their structural and energetic stability over 100 ns.

Methods: Five acrylamide-based polymers were investigated using AutoDock Vina for molecular docking and Gromacs for molecular dynamics simulations, focusing on binding affinities, hydrogen bonds, hydrophobic interactions, and complex behaviors over 100 ns.

Results: Acetoacetate binds well to the polymers PAM2 and PAM5, with the maximum binding affinity being 2.738 and 2.49 kcal/mol, respectively. PAM1, PAM3, and PAM4 had significant binding affinities; however, PAM4 had a lesser binding affinity of-1.534 kcal/mol, making it less appropriate for acetoacetate-specific MIP applications. The molecular dynamics investigation discovered that PAM5 had the best total energy, indicating a relatively stable interaction environment.

Conclusion: The study reveals PAM5 as a promising candidate with high binding affinity and multiple hydrogen bonds with acetoacetate, providing insights for acetoacetate-specific MIP design and molecular recognition progress.

Keywords: Acetoacetate, Acrylamide-based polymers, MIPs, Molecular docking, Molecular dynamics

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INTRODUCTION

Acetoacetate plays a significant role in diabetes, specifically in the context of type 1 diabetes and diabetic ketoacidosis (DKA) [1]. Type 1 diabetes is an autoimmune condition in which the body's immune system attacks and destroys the insulin-producing beta cells in the pancreas [2]. As a result, individuals with type 1 diabetes have little to no insulin production, leading to high blood glucose levels. In the absence of sufficient insulin, the body cannot utilize glucose effectively for energy [3]. As an alternative energy source, the liver begins to break down fats into ketone bodies, including acetoacetate, β-hydroxybutyrate, and acetone [4]. This process is called ketogenesis. On the other hand, DKA is a severe and potentially life-threatening complication that can occur in individuals with type 1 diabetes. It typically develops when blood glucose levels are extremely high and there is a significant shortage of insulin in the body. Without insulin to facilitate glucose uptake by cells, the body resorts to breaking down fats for energy, resulting in excessive production of ketone bodies [5].

Acetoacetate, being one of the ketone bodies, accumulates in the blood, leading to a condition known as ketoacidosis [6]. The presence of high levels of acetoacetate and other ketone bodies lowers blood pH, causing it to become acidic. This acidic environment can disrupt normal physiological processes and lead to various symptoms, including deep and rapid breathing (Kussmaul breathing), fruity-smelling breath, nausea, vomiting, abdominal pain, and altered mental status [7]. In clinical settings, acetoacetate levels in the blood and urine can be monitored as an indicator of ketosis and DKA in individuals with type 1 diabetes [8]. Measuring ketone levels helps healthcare professionals assess the severity of the condition and guide appropriate treatment strategies, which often include intravenous fluids, insulin therapy, and correction of electrolyte imbalances.

The most common method is through the measurement of ketone bodies in the blood, which includes acetoacetate and beta-hydroxybutyrate [9]. Acetoacetate levels can also be detected in the urine, although this method is not as precise as blood testing [10]. Urine test strips, known as ketone test strips, are used to check for the presence of ketones in the urine. These strips change color based on the amount of acetoacetate present in the urine, indicating the level of ketosis. It's important to note that urine ketone testing may not provide real-time information about the current state of ketosis since ketones in the urine are a reflection of past ketone levels in the blood. Additionally, as the body becomes adapted to using ketones for fuel, fewer ketones may be excreted in the urine, leading to potentially misleading results.

Molecularly Imprinted Polymers (MIPs) have shown significant promise for various applications in diabetes management and treatment [11]. MIPs are synthetic polymers that possess specific binding sites or "imprints" that are designed to selectively recognize and bind to target molecules with high affinity and specificity [12]. This molecular recognition property makes MIPs well-suited for various biomedical and pharmaceutical applications, including diabetes-related ones. MIPs can be used to develop highly sensitive and specific glucose sensors for continuous glucose monitoring (CGM) in diabetes patients [13]. The MIP-based glucose sensors can detect and measure glucose levels in the blood or interstitial fluid, providing valuable information for diabetes management, insulin dosing, and lifestyle adjustments.

MIPs can be designed to recognize specific diabetes-related biomarkers, such as glycosylated hemoglobin (HbA1c) or other indicators of diabetes complications [14]. MIP-based biosensors can detect and quantify these biomarkers in biological samples, aiding in disease diagnosis, monitoring, and management [15]. While the potential applications of MIPs in diabetes are promising, further research and development are needed to translate these concepts into practical and clinically applicable solutions. Challenges include optimizing the imprinting process, improving the stability, biocompatibility, and scalability of MIPs, as well as conducting extensive *in vivo* studies to validate their safety and efficacy.

Molecular docking and molecular dynamics simulations play crucial roles in the design and development of MIPs [16]. These computational techniques provide valuable insights into the interactions between template molecules (molecular targets) and functional monomers during the MIP synthesis process. By employing these tools, researchers can optimize the imprinting process, improve the selectivity and affinity of MIPs, and guide the rational design of MIPs for specific applications. Docking provides initial binding configurations, and MD simulations offer a dynamic view of the binding process, taking into account the flexibility and adaptability of the MIP structure. By iteratively combining docking and MD simulations, researchers can refine and validate the binding interactions between the template and functional monomers, ensuring that the designed MIP has the desired specificity and selectivity. This integrated approach facilitates the development of high-performance MIPs for various applications.

Acrylamide-based monomers offer several advantages over other monomers when used in Molecularly Imprinted Polymers (MIPs) for various applications. Acrylamide monomers have been shown to provide selectivity to MIP materials towards both carboxylic anion targets and more complex species, such as proteins [17]. This enhanced selectivity makes them suitable for applications requiring specific binding and recognition. Acrylamide monomers, with their weakly acidic nature, exhibit stronger hydrogen-bonding interactions with certain target molecules, such as histamine, compared to other monomers like methacrylic acid (MAA). This property contributes to the improved binding affinity and selectivity of acrylamide-based MIPs. MIPs, including those using acrylamide-based monomers, are known for their fast and low-cost synthesis, making them efficient and practical for various applications, such as solid-phase extraction (SPE) and high-performance liquid chromatography (HPLC) [18]. Acrylamide-based MIPs can be synthesized and characterized using computational predictions and experimental techniques, making them suitable for advanced research and development in the field of MIPs [19].

The study in molecular docking and molecular dynamics studies for acetoacetate in polyacrylamide-based polymer (PAM) is the lack of comprehensive understanding of their molecular interaction. Investigating the specific binding sites and mechanisms of acetoacetate in acrylamide-based polymers. The study can be expanded to identify the key functional groups in the polymer that are responsible for the interaction with acetoacetate. This information can provide insights into the design and optimization of acrylamide-based polymers for various applications. Exploring the effect of polymer structure and composition on the binding affinity and stability of acetoacetate. By studying a range of acrylamide-based polymers with different structures and compositions, we can gain a better understanding of how these factors influence the interaction with acetoacetate. This knowledge can be used to tailor the properties of the polymers for specific applications.

Therefore, the present study aims to address this research gap through molecular docking analyses to explore binding affinities of acetoacetate in acrylamide-based polymers (pam) for MIPs via a molecular docking and molecular dynamics study. The study investigates the binding affinity of acetoacetate, a key molecule involved in the MIPs process, with different types of polymers. Molecular docking using AutoDock Vina was employed to predict and compare the binding affinities of acetoacetate with PAM1, PAM2, PAM3, PAM4, and PAM5 polymers. By addressing these research gaps, the study can contribute to a more comprehensive understanding of the interaction between acetoacetate and acrylamide-based polymers, enabling the development of improved materials for various applications.

MATERIALS AND METHODS

Materials

The workstation was equipped with a double processor of Intel® Xeon E5-2673v2 20 core 40 Thread 2.3 Ghz, 64 GB of RAM, and RTX 4060 Ti with dual operating system: Windows 10 Pro-64-bit and Ubuntu 22 for molecular docking and molecular dynamics simulation.

Molecular docking simulation

Preparation of molecular structures

A set of PAMs were chosen as potential functional monomers for imprinting acetoacetate (table 1). The 3D structures of acetoacetate and PAMs were generated and optimized the geometry using Avogadro software [20]. The selection criteria included the presence of specific functional groups capable of forming hydrogen bonds and hydrophobic interactions with the target molecule.

Table 1: The specific functional groups of Acrylamide-based polymers (PAM) and acetoacetate

Polymer	Monomer	Monomer ID	R_1	R_2
PAM1	Acrylamide	Aam	-H	-H
PAM2	*N*-(Hydroxymethyl)acrylamide	NHMAm	$-CH_2-OH$	-H
PAM3	*N*-(Hydroxyethyl)acrylamide	NHEAm	$-CH_2-CH_2-OH$	-H
PAM4	*N*,*N*-Dimethylacylamide	DMAm	$-CH_3$	$-CH_3$
PAM5	*N*-[Tris(hydroxymethyl)methyl]acrylamide	TrisNHMAm	$-C-(CH_2-OH)_3$	-H

Grid generation for docking

A docking grid was created to define the search space around the functional monomer (receptor) for acetoacetate (ligand) docking. The grid encompassed the entire polymer structure and allowed for efficient exploration of potential binding sites.

Molecular docking with AutoDock Vina

The prepared polymers (PAM) and ligand (acetoacetate) structures were used as input for the AutoDock Vina software [21]. The molecular docking simulations were performed using the Lamarckian Genetic Algorithm, which explored various conformations of the ligand within the binding site to find the most energetically favorable binding pose [22–25].

Analysis of docking results

After docking simulations were completed, the output files were analyzed to identify the binding poses with the lowest binding affinity scores (kcal/mol). The energy of the docked complexes was analyzed using Discovery Studio Visualizer [26]. The docking poses with the highest negative binding energy represented the most

stable and favorable interactions between acetoacetate and the PAM polymers.

Molecular dynamics simulation

In pursuit of understanding the stability of acetoacetate complexes within different polymer matrices for MIPs, a comprehensive molecular dynamics (MD) simulation for 100 ns was employed using the GROMACS software [27]. ACPYPE, a tool based on ANTECHAMBER was used for generating automatic topologies and parameters for molecular mechanics programs [28].

System preparation

The acetoacetate-MIP complexes for each polymer (PAM1, PAM2, PAM3, PAM4, and PAM5) were built through molecular docking. The complexes were placed within a simulation box filled with an appropriate solvent (e.g., water) to mimic the surrounding environment. Counterions were added to neutralize the system's charge. The system underwent energy minimization to remove steric clashes and unfavorable contacts.

Equilibration

The system was equilibrated under constant volume and temperature (NVT) conditions. Temperature coupling and pressure coupling algorithms were applied to achieve the desired conditions. The equilibrated system was further equilibrated under constant pressure and temperature (NPT) conditions to allow for density equilibration.

Production

The equilibrated system was subjected to a production MD run. The GROMACS software, utilizing force fields and appropriate simulation parameters, was used to conduct the simulation. The time step, number of steps, and integration algorithms were specified. Trajectories were saved at regular intervals to record the system's coordinates, velocities, and energies throughout the simulation.

Data analysis

The RMSD of the complex structures was calculated concerning the initial structure to assess structural stability. The radius of gyration of the complex was computed to understand the compactness and flexibility of the system. Solvent Accessible Surface Area (SASA) was determined to analyze the surface exposure of the complex to the solvent environment. The ΔTOTAL values of energy components, including ΔVDWAALS, ΔEEL, ΔEGB, ΔESURF, ΔGGAS, and ΔGSOLV, were calculated to evaluate the complex's energetic stability.

RESULTS

Binding mode interaction

Molecular docking is a valuable tool in drug discovery that provides valuable data on the binding affinity, hydrogen bonding, and hydrophobic interactions between the acetoacetate with the studied polymers. The results obtained from molecular docking simulations can be used to identify potential polymer candidates and predict binding affinities of acetoacetate with the studied polymers (fig. 1 and 2).

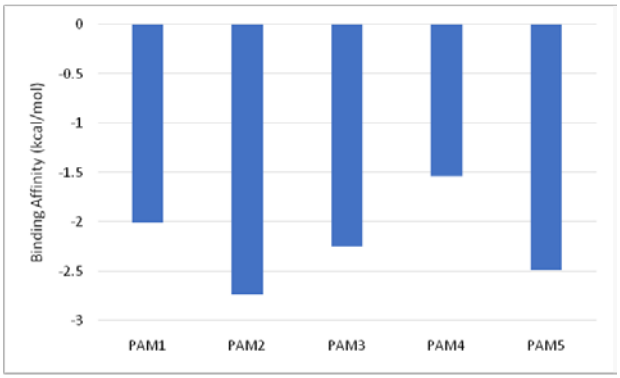


Fig. 1: The binding affinity of acetoacetate during its contact with a variety of polymers based on acrylamide (PAM)

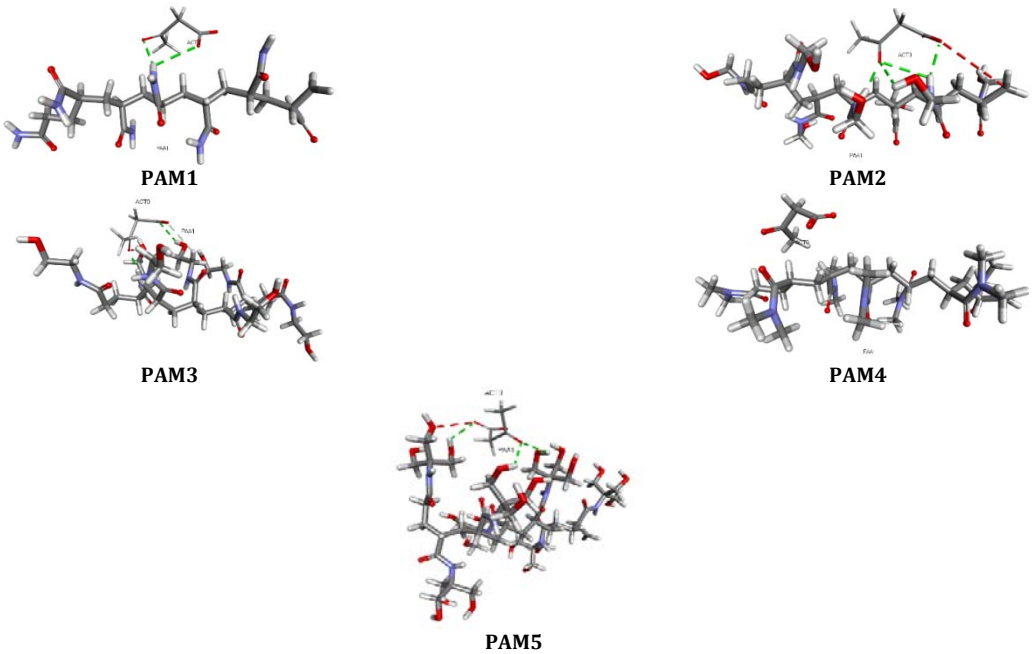


Fig. 2: Acetoacetate's three-dimensional interaction with a variety of PAMs. (The green dotted lines are hydrogen bonds)

Among the polymers studied, PAM2 demonstrated the highest binding affinity with acetoacetate, recording a value of -2.738 kcal/mol. This suggests that PAM2 was particularly adept at forming stable and energetically favorable complexes with acetoacetate, indicating its strong potential for MIPS applications where robust molecular interactions are essential [29]. The presence of multiple hydrogen bonds in PAM2 (5 hydrogen bonds) (fig. 2) further strengthens its affinity for acetoacetate. Hydrogen bonds play a critical role in stabilizing molecular complexes, and the abundance of hydrogen bonds in PAM2 indicates a strong and specific binding interaction with acetoacetate.

PAM1, PAM3, and PAM5 (fig. 2) also displayed significant binding affinities, recording values of-2.012 kcal/mol,-2.248 kcal/mol, and-2.490 kcal/mol, respectively. The formation of hydrogen bonds in these polymers (2 hydrogen bonds in PAM1 and 4 hydrogen bonds in PAM5) further reinforces their potential for acetoacetate binding in MIPS systems. On the other hand, PAM4 exhibited a relatively weaker binding affinity with acetoacetate, recording a value of-1.534 kcal/mol. The absence of any hydrogen bonds or hydrophobic interactions between PAM4 and acetoacetate suggests that it might have limited suitability for MIPS applications involving acetoacetate binding.

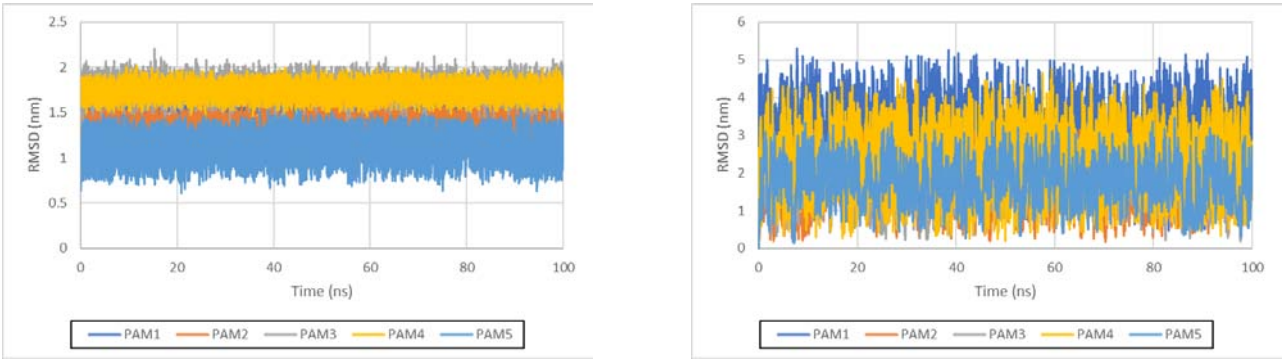
The stability of a bond increases with negative energy in the interaction, as energy is released during bond formation, enhancing the stability of the system [30]. In the case of acetoacetate and acrylamide-based polymers (MIPs), the strong and energetically favorable complexes formed between the two indicate the stability of the bond and the potential for robust molecular interactions.

Stability of the binding interactions

Molecular dynamics simulations can be used to systematically analyze the stability of various ligand poses under simulation. The number of hydrogen bonds and other interactions between the ligand and protein can be monitored during molecular dynamics simulations to assess the stability of the binding interactions.

RMSD

We will analyze the fluctuations in Root Mean Square Deviation (RMSD) over a 100 ns simulation for different polymers: PAM1, PAM2, PAM3, PAM4, and PAM5 (fig. 3). RMSD is a measure of how much the atomic positions of a molecule deviate from a reference structure over time. In our study, we conducted a comprehensive analysis of five different polymers, PAM1, PAM2, PAM3, PAM4, and PAM5, to understand their behavior and interactions through the lens of RMSD fluctuations. The RMSD values served as crucial indicators of how these polymers responded to external influences and underwent structural changes throughout a simulation.



PAM **Acetoacetate**

Fig. 3: Movement in the MD simulation for 100 ns of acetoacetate and acrylamide-based polymers (PAM)

On average, PAM1 exhibited an RMSD fluctuation of approximately 1.65 Å, suggesting a moderate degree of structural variability. The highest observed RMSD value was around 2.00 Å, indicating a moment of notable conformational change, while the lowest value hovered around 1.34 Å, signifying a period of relatively enhanced stability. In the case of PAM2, the average RMSD fluctuation was approximately 1.32 Å, suggesting a relatively stable response to external factors. The highest RMSD value observed was roughly 1.70 Å, reflecting a moderate conformational shift, while the lowest value recorded was approximately 0.90 Å, pointing to instances of strong stability [31].

PAM3 exhibited an average RMSD fluctuation of about 1.78 Å, indicating a moderate level of structural dynamics. The highest RMSD value observed was approximately 2.20 Å, suggesting significant conformational changes, while the lowest value was around 1.35 Å, implying periods of enhanced stability. For PAM4, the average RMSD fluctuation stood at around 1.74 Å, indicating noticeable structural changes. The top RMSD value observed was approximately 2.02 Å, signifying significant conformational fluctuations, while the lowest value recorded was about 1.44 Å, indicating a relatively stable configuration. Lastly, PAM5 displayed an average RMSD fluctuation of around 1.10 Å, implying moderate structural dynamics. The highest RMSD value observed was approximately 1.54 Å, suggesting notable conformational changes, while the lowest value recorded was approximately 0.61 Å, indicating periods of strong stability.

Comparing the polymers, it's evident that PAM1, PAM2, and PAM3 generally had lower RMSD values and exhibited moderate fluctuations, implying a relatively stable conformation. On the other hand, PAM4 and PAM5 showed higher RMSD values, indicating more substantial structural changes or flexibility. These fluctuations in RMSD reflect the inherent characteristics and behavior of each polymer based on their chemical structures and functional groups. The differences in polarity introduced by the various monomers likely contributed to the observed variations in structural stability and flexibility.

Analyzing the RMSD data, we gained valuable insights into the dynamic nature of the acetoacetate interactions within each polymer: The RMSD values for acetoacetate in PAM1 started at 3.83 Å, indicating an initial deviation from the reference structure. Throughout the simulation, the RMSD values experienced fluctuations, with occasional increases and decreases, suggesting moderate flexibility and adjustments in the acetoacetate's conformation. acetoacetate in PAM2 exhibited a distinct behavior with an initial RMSD of only 0.00055 Å. This exceptionally low value suggests a strong and stable interaction between acetoacetate and the PAM2 polymer. As the simulation progressed, the RMSD values increased slightly, indicating some degree of structural adaptation.

RMSD values for acetoacetate in PAM3 began at 0.00079 Å, similar to PAM2. However, over time, the values increased, implying that acetoacetate in PAM3 experienced more fluctuations and conformational changes compared to PAM2. Acetoacetate in PAM4 started with an initial RMSD of 0.00064 Å, similar to PAM2 and PAM3. The RMSD values then increased significantly at certain time points, particularly at 5 ns and 45 ns, indicating structural rearrangements or transitions in the acetoacetate-PAM4 complex. Acetoacetate's RMSD values in PAM5 began at 0.00050 Å, akin to the other low initial RMSD values. Over the simulation, the RMSD values

showed periodic increases and decreases, reflecting dynamic interactions between acetoacetate and the PAM5 polymer.

Comparing the polymers, it's evident that PAM2 exhibited the most stable interaction with acetoacetate, as indicated by the consistently low RMSD values. PAM3 and PAM5 also maintained relatively stable interactions, albeit with some fluctuations. On the other hand, PAM1 and PAM4 displayed higher RMSD values, suggesting more pronounced structural changes and flexibility in the acetoacetate interactions within these polymers.

Gyration

We embarked on an investigation into the interactions of acetoacetate within a spectrum of polymer environments, encompassing PAM1, PAM2, PAM3, PAM4, and PAM5 (fig. 4). The central objective of this exploration was to glean insights into how these diverse polymer matrices influenced the behavior of acetoacetate, elucidated through the lens of gyration fluctuations observed over a 100,000 ps simulation period.

The average gyration fluctuations for acetoacetate in PAM1 were calculated at approximately 0.543. These values represented the general tendency of acetoacetate to exhibit relatively stable interactions within this polymer environment. The highest gyration fluctuation recorded was around 0.597, signifying instances of moderate conformational shifts, while the lowest gyration value observed was approximately 0.415, suggesting periods of enhanced structural stability.

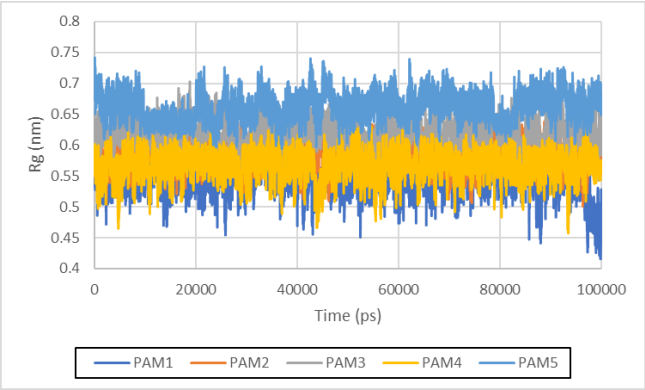


Fig. 4: Gyration fluctuations for acetoacetate in polymers based on acrylamide (PAM) were detected throughout 100,000 ps during the simulation

In the case of PAM2, acetoacetate's average gyration fluctuations were computed to be approximately 0.589. This indicated that acetoacetate displayed consistent and relatively stable behavior within the polymer matrix. The highest gyration value reached around 0.650, indicating moderate deviations from the reference conformation, while the lowest gyration value noted was roughly 0.501, indicating substantial stability.

The average gyration fluctuations for acetoacetate within PAM3 amounted to approximately 0.617. This average value suggested a moderate level of structural dynamics, with the highest gyration value recorded at around 0.703, indicating notable conformational changes, and the lowest value at approximately 0.533, implying moments of enhanced structural stability.

In the context of PAM4, acetoacetate displayed average gyration fluctuations of approximately 0.568. These values denoted noticeable changes in acetoacetate's structural arrangement within the polymer. The highest gyration value reached approximately 0.638, signifying significant conformational variability, while the lowest gyration value observed was around 0.458, implying relatively stable conformations.

Lastly, within the PAM5 environment, acetoacetate exhibited average gyration fluctuations of approximately 0.663. This highlighted the moderate structural dynamics experienced by acetoacetate within this polymer matrix. The highest gyration value recorded was about 0.742, indicating substantial conformational shifts, while the lowest value observed was approximately 0.594, indicating intervals of robust stability.

SASA

Analyzing the SASA fluctuations data yielded insightful revelations into the dynamic interplay between acetoacetate and each polymer (fig. 5). The average SASA fluctuations for acetoacetate within PAM1 were computed at approximately 8.012. These values indicated the overall trend of acetoacetate exhibiting relatively stable interactions with the surrounding solvent and the polymer. The highest SASA fluctuation recorded was around 8.864, signifying instances of increased surface exposure and potential interactions, while the lowest SASA value observed was approximately 6.9, implying periods of reduced solvent accessibility.

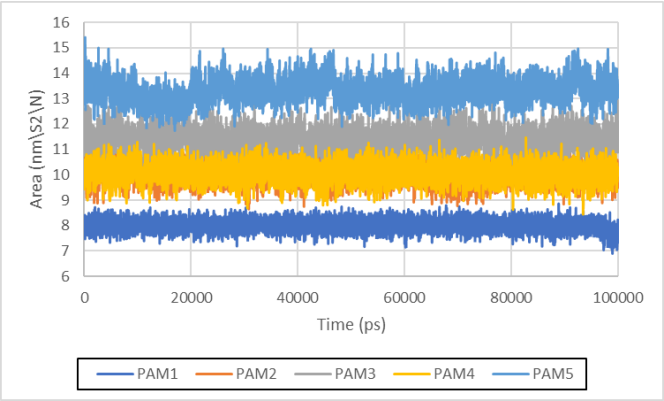


Fig. 5: SASA fluctuations for acetoacetate in polymers based on acrylamide (PAM) were detected for 100,000 ps during the simulation

In the case of PAM2, the average SASA fluctuations for acetoacetate were calculated to be around 10.003. This highlighted the tendency of acetoacetate to maintain relatively stable surface accessibility within this polymer matrix. The highest SASA value reached around 11.266, indicating moderate fluctuations in surface exposure, while the lowest SASA value noted was roughly 8.651, suggesting periods of decreased solvent accessibility.

Acetoacetate's average SASA fluctuations within PAM3 amounted to approximately 11.383. This average value suggested moderate variations in the extent of surface exposure and interaction between acetoacetate and the solvent or polymer matrix. The highest SASA value recorded was around 13.088, indicating notable fluctuations in surface accessibility, and the lowest value at approximately 9.779, implying relatively stable periods of interaction.

Within the PAM4 environment, the average SASA fluctuations for acetoacetate were approximately 10.076. This signified noticeable changes in the extent of surface accessibility and solvent interaction. The highest SASA value reached approximately 11.466, pointing to significant surface fluctuations, while the lowest SASA value observed was around 8.463, indicating periods of relatively reduced interaction with the surrounding environment.

Finally, acetoacetate within the PAM5 environment exhibited average SASA fluctuations of approximately 13.341. This emphasized the dynamic and fluctuating nature of acetoacetate's surface interactions within this polymer matrix. The highest SASA value recorded was about 15.414, indicating substantial surface exposure and dynamic interaction, while the lowest value observed was approximately 11.748, suggesting periods of decreased surface accessibility.

Energy component

We conducted a comprehensive investigation into the interactions between acetoacetate and a range of polymer environments, including PAM1, PAM2, PAM3, PAM4, and PAM5. Our primary focus was to analyze and compare the various energy components that contribute to these interactions, specifically ΔVDWAALS, ΔEEL, ΔEGB, ΔESURF, ΔGGAS, ΔGSOLV, and ΔTOTAL values (fig. 6).

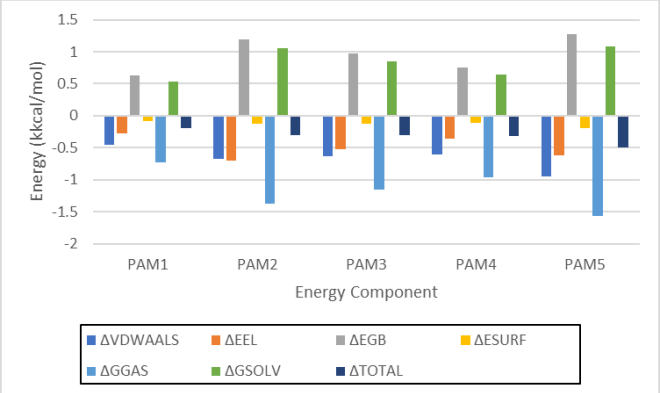


Fig. 6: Acetoacetate energy component in acrylamide-based polymers (PAM) for the MMGBSA simulation

Across all polymer environments, acetoacetate exhibited attractive van der Waals interactions. The magnitudes of ΔVDWAALS ranged from-0.45 to-0.95 kcal/mol. PAM5 demonstrated the strongest van der Waals interaction, indicating a higher tendency for acetoacetate to form these attractive forces in this polymer. The electrostatic interactions, represented by ΔEEL, played a crucial role in acetoacetate's behavior within the polymer matrices. The values of ΔEEL ranged from 0.7 to 0.36 kcal/mol, suggesting predominantly attractive electrostatic interactions. PAM2 displayed the most favorable electrostatic interactions, while PAM4 exhibited the least favorable.

Solvation energy, ΔEGB, captured the energy change upon transferring acetoacetate from vacuum to solvent. Positive ΔEGB values indicated acetoacetate's preference for solvation. The range of ΔEGB values was from 0.63 to 1.28 kcal/mol, with PAM5 showing the highest preference for solvation. The surface energy component, ΔESURF, indicated the energy change when acetoacetate was exposed to the polymer surface. The fluctuations in ΔESURF were relatively small across all polymers, suggesting minor variations in surface interactions.

The Gibbs free energy of solvation, ΔGGAS, encompassed the overall energetic favorability of acetoacetate's dissolution. Negative ΔGGAS values indicated favorable dissolution in all polymer environments, with PAM5 exhibiting the most favorable dissolution. The change in solvation-free energy, ΔGSOLV, indicated the overall favorability of acetoacetate's solvation. Positive ΔGSOLV values demonstrated the stabilization of acetoacetate by solvent interactions. PAM5 had the most favorable solvation environment for acetoacetate.

The cumulative effect of all energy components, ΔTOTAL, offered insights into the net energy change associated with acetoacetate's interactions. The ΔTOTAL values were negative across all polymers, indicating energetically favorable interactions. PAM5 displayed the most favorable ΔTOTAL, suggesting a particularly stable interaction environment.

DISCUSSION

Molecular docking studies using acrylamide-based polymers for molecularly imprinted polymers (MIPs) have been conducted in various fields, including glucose monitoring, drug delivery, and protein detection. The acrylamide-based monomers that were smaller in size and that formed comparatively fewer hydrogen bonds with the template molecule resulted in better MIPs for cardiovascular disease testing [32]. The other study introduced a molecular mechanics-based multi-monomer simultaneous docking approach (MMSD) to computationally screen monomer combinations for selective MIPs for myoglobin [33]. These findings highlight the versatility and potential of MIPs for various applications.

PAM1, made from acrylamide monomers, had a moderate polarity due to the presence of an amide functional group, allowing for some interaction with polar substances [34]. PAM2, synthesized using *N*-(Hydroxymethyl)acrylamide, exhibited higher polarity than PAM1 because of the hydroxymethyl group, which increased the potential for hydrogen bonding and polar interactions[1][4]. PAM3, which utilized *N*-(Hydroxyethyl)acrylamide, displayed even greater polarity than PAM2 due to the larger hydroxyethyl group that facilitated stronger interactions with polar solvents[1]. PAM4, formed from *N*, *N*-Dimethylacylamide, had reduced polarity compared to PAM1, as the dimethyl groups hindered hydrogen bonding and decreased interaction with polar compounds. PAM5, derived from *N*-[Tris(hydroxymethyl)methyl]acrylamide, exhibited the highest polarity among the polymers due to the multiple hydroxymethyl groups, enabling extensive interactions with polar molecules. Overall,

the polarity of these polymers varied based on the chemical structures and functional groups of their respective monomers, ranging from moderate to high polarity, with PAM5 being the most polar due to its complex hydroxymethyl arrangement.

Analyzing the relationship between the functional groups of PAMs and the binding energy (kcal/mol) from molecular docking results can provide insights into the interactions that contribute to the strength of binding between the polymer and the target molecule. PAM1 represents a generic Acrylamide-based Polymer. The binding energy of-2.012 kcal/mol suggests that the polymer as a whole has some interaction with the target molecule, but the absence of specific functional groups like hydroxyl or amide groups might limit the strength of the interaction. PAM2, composed of Acrylamide (Aam), exhibits a relatively high binding energy of-2.738 kcal/mol. The vinyl group in acrylamide's structure could engage in van der Waals interactions and potentially form hydrogen bonds, resulting in a favorable binding energy. This indicates that the vinyl group is playing a role in stabilizing the complex.

PAM3, which consists of *N*-(Hydroxymethyl)acrylamide (NHMAm), shows a moderate binding energy of-2.248 kcal/mol. The hydroxymethyl group in NHMAm contributes to hydrogen bonding interactions, enhancing the affinity between the polymer and the target. However, the relatively lower binding energy compared to PAM2 might indicate that other factors or groups are also influencing the interaction. PAM4, containing *N*-(Hydroxyethyl)acrylamide (NHEAm), displays a lower binding energy of-1.534 kcal/mol. The hydroxyethyl group in NHEAm is longer and more flexible than the hydroxymethyl group, potentially influencing the binding mode. The lower binding energy could be due to the flexibility of the hydroxyethyl group, affecting the optimal binding conformation. PAM5, comprising *N*,*N*-Dimethylacylamide (DMAm), shows a binding energy of-2.490 kcal/mol. The amide group in DMAm is likely forming hydrogen bonds and van der Waals interactions with the target molecule. The presence of the amide group can contribute to a stable and specific binding mode. Amide groups, hydroxyl groups, and carboxyl groups are involved in hydrogen bonding and electrostatic interactions with the target molecule [35].

Analyzing the relationship between the functional groups of Acrylamide-based Polymers (PAM) and the energy component from MD results can provide insights into the stability and dynamics of the polymer-target interactions. PAM1, representing a generic Acrylamide-based Polymer, exhibits a relatively low energy component of-0.19 kcal/mol. This suggests that the polymer as a whole has a relatively weak interaction with the target molecule. The absence of specific functional groups might limit the strength of the interactions, leading to a less favorable energy component. PAM2, composed of Acrylamide (Aam), shows a slightly higher energy component of-0.31 kcal/mol. The vinyl group in acrylamide might be contributing to van der Waals interactions and hydrogen bonding, leading to a relatively stable interaction with the target. This suggests that the vinyl group is influencing the overall energy of the system. PAM3, containing *N*-(Hydroxymethyl)acrylamide (NHMAm), displays a similar energy component of-0.3 kcal/mol. The hydroxymethyl group in NHMAm could be forming hydrogen bonds and van der Waals interactions, contributing to a stable interaction. The energy component is relatively consistent with PAM2, indicating that both vinyl and hydroxymethyl groups play comparable roles.

PAM4, which incorporates *N*-(Hydroxyethyl)acrylamide (NHEAm), shows a slightly higher energy component of-0.32 kcal/mol. The hydroxyethyl group in NHEAm, being longer and more flexible, might influence the conformation and dynamics of the interaction, potentially leading to a slightly less favorable energy component. PAM5, comprising *N*,*N*-Dimethylacylamide (DMAm), displays the highest energy component of-0.49 kcal/mol. The amide group in DMAm is likely forming stable hydrogen bonds and van der Waals interactions with the target molecule. The presence of the amide group contributes to a relatively strong and specific binding interaction, resulting in a more favorable energy component.

The relatively low energy component suggests that PAM1 has weak interactions with the target, resulting in less stability in the MD simulations. The corresponding low binding energy from docking indicates a relatively weaker interaction between PAM1 and the target molecule. PAM2 exhibits a higher energy component and a higher binding energy compared to PAM1. The presence of the vinyl group in acrylamide likely contributes to van der Waals and hydrogen bonding interactions, resulting in a more stable binding mode. PAM3 shows similar energy and binding energies as PAM2. The hydroxymethyl group in NHMAm could be participating in hydrogen bonding interactions, enhancing the affinity of the binding. PAM4 displays a relatively low energy component and binding energy. The longer and more flexible hydroxyethyl group might affect the conformation and dynamics of the interaction, leading to a less favorable binding mode. PAM5 exhibits the highest energy and binding energies among the PAM variants. The presence of the amide group in DMAm likely contributes to strong hydrogen bonding and van der Waals interactions, resulting in a stable and specific binding mode.

Analyzing the relationship between the functional groups of Acrylamide-based Polymers (PAM) and the energy component from molecular dynamics (MD) for MIPs in diabetes can provide insights into the structure-function relationships of these polymers. Functional polymers bear specified chemical groups and have specified properties [36]. In the context of MIPs for diabetes, these functional groups can play a crucial role in the selective recognition and binding of glucose or other relevant biomolecules. The other study about the Strategies for Molecularly Imprinted Polymer Development [37] demonstrated the use of MAM-based MIPs for static and dynamic binding and selectivity tests with ACET, BENZ, and PIV solutions. Although not directly related to diabetes, this study highlights the potential of functional groups in MIPs for specific binding and selectivity. The study by using an MD and QM/MM investigation of acrylamide-based leads to target the main protease of SARS-CoV-2 [38] explored the potential of acrylamide-based molecules as covalent inhibitors. While not directly related to diabetes, this study demonstrates the versatility of acrylamide-based polymers in various applications, including drug targeting and binding. In conclusion, these functional groups play a crucial role in the selective recognition and binding of biomolecules, as demonstrated in various studies on MIPs for different applications.

CONCLUSION

The binding energies observed in the molecular docking results suggest that functional groups, such as hydroxyl and amide groups, play a crucial role in the interactions between Acrylamide-based Polymers (PAM) and acetoacetate. These groups contribute to hydrogen bonding and van der Waals interactions, influencing the strength and specificity of the binding. Overall, based on the results of molecular docking simulations, acetoacetate was able to interact with all Acrylamide-based Polymers (PAM). However, after being elaborated with molecular dynamics simulations, acetoacetate forms the most stable bond with PAM5.

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AUTHORS CONTRIBUTIONS

All the authors have equally contributed to the current study.

CONFLICT OF INTERESTS

All the authors declare no conflicts of interest.

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