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Poly (PEGDMA-MAA) copolymeric micro and nanoparticles for oral insulin delivery : A molecular mechanistic revisit

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Abstract - Poly(ethylene glycol) dimethacrylate (PEGDMA) and methacrylic acid (MAA) based micro and nanoparticles were prepared for oral insulin delivery. The reactional profiles of MAA, PEGDMA and PEGDMA-MAA were elucidated using molecular mechanics energy relationships (MMER) in vacuum and in a solvated system by exploring the spatial disposition of different concentrations of polymers with respect to each other. Furthermore, the incorporation of insulin within the polymeric matrix was modeled using Connolly molecular surfaces. The computational results corroborated with the experimental and analytical data. The ability to effectively control blood glucose level coupled with the non-toxic behavior of the nanoparticles renders them a potential candidate for insulin delivery.

Keywords: *pH sensitive copolymer, molecular mechanics energy relationship, oral insulin delivery, micro and nanoparticles.*

I. INTRODUCTION

Administering drug orally is by far the most widely used route of administration, although it is generally not feasible for peptide and protein drugs. Insulin - a peptide - is the most effective and widely used drug in the treatment of advanced-stage diabetes. Development of non-invasive insulin delivery systems has always been a major challenge for the treatment of diabetes. Insulin administration through the oral route passes through the hepatic pass and produces the similar effect as pancreas-secreted insulin [1]. The key reasons for the low oral bioavailability of insulin are: acidic pH, presystemic enzymatic degradation and its poor penetration through the intestine membrane. Various strategies developed to try and achieve effective oral insulin delivery include: co-administration with absorption enhancers [2–3], enzyme inhibitors [3–4], polymeric carriers [5–7], and lipid based carriers as liposomes [8]. Interpolymer complex formation of high molecular weight polyethylene glycol (PEG) and polycarboxylic polymers like polymethacrylic acid (PMAA) and polyacrylic acid (PAA) is well documented [9].

The present *in silico* studies were conducted on pH sensitive polymeric micro and nanoparticles synthesized and reported earlier by our group to evaluate the effect

of particle size on loading and release of insulin under various physiological relevant pH conditions [10].

II. COMPUTATIONAL METHODS

Static lattice atomistic simulations

Molecular simulations were performed using commercial softwares: HyperChemTM 8.0.8 Molecular Modeling System (Hypercube Inc., Gainesville, Florida, USA) and ChemBio3D Ultra 11.0 (Cambridge Soft Corporation, Cambridge, UK). The structures of PEGDMA and MAA were built in their syndiotactic stereochemistry as 3D models using ChemBio3D Ultra while the structure of insulin active sequence was generated using built-in sequence editor module of HyperChem. The models were energy-minimized using a progressive-convergence-strategy where initially the MM+ force field was used followed by energy-minimization using the Amber 3 (Assisted Model Building and Energy Refinements) force field. The conformer having the lowest energy was used to create the polymer-polymer and polymer-protein complexes. A complex of one polymer molecule with another was assembled by disposing the molecules in a parallel way, and the same procedure of energy-minimization was repeated to generate the final models: MAA, PEGDMA, MAA-PEGDMA and PEGDMA-MAA-insulin. Full geometry optimization was carried out in vacuum

employing the Polak–Ribiere conjugate gradient algorithm until an RMS gradient of 0.001kcal/mol was reached. For molecular mechanics calculations in vacuum, the force fields were utilized with a distance-dependent dielectric constant scaled by a factor of 1. The 1-4 scale factors were electrostatic 0.5 and van der Waals 0.5 [11].

Molecular mechanics assisted model building and energy refinements

A molecular mechanics conformational searching procedure was employed to acquire the data employed in the statistical mechanics analysis, and to obtain differential binding energies of a Polak–Ribiere algorithm and to potentially permit application to polymer composite assemblies. MM+ is a HyperChem modification and extension of Norman Allinger's Molecular Mechanics program MM2 [12], whereas AMBER is a package of computer programs for applying molecular mechanics, normal mode analysis, molecular dynamics and free energy calculations to simulate the structural and energetic properties of molecules [13].

Molecular dynamics simulations (MDS)

The polymer chains initially minimized by molecular mechanics were then minimized by molecular dynamics for 1.0ps (time step = 0.001ps) at 300K with the Nose–Hoover thermostat. For evaluation of the stability of a simulation and the extent of equilibration and for identification of the interesting low energy conformations, molecular dynamics calculations were averaged and saved as kinetic energy (EKIN), potential energy (EPOT), total energy (ETOT) and temperature (TEMP). Equilibrium was established before recording the measurements wherein the instantaneous potential and kinetic energy were monitored to determine when the system reaches equilibrium. Thereafter, the simulation was allowed to run for 1000 time-steps before taking measurements [14].

III. RESULTS AND DISCUSSION

MMER analysis

Molecular mechanics energy relationship (MMER), a method for analytico-mathematical representation of potential energy surfaces, was used to provide information about the contributions of valence terms, noncovalent coulombic terms and noncovalent van der Waals interactions for polymer/polymer/bioactive complexes.

The MMER model for potential energy factor in various molecular complexes can be written as:

$$E_{\text{molecule/complex}} = V_{\Sigma} = V_b + V_{\theta} + V_{\varphi} + V_{ij} + V_{hb} + V_{el} \dots(1)$$

where, V_{Σ} is related to total steric energy for an optimized structure, V_b corresponds to bond stretching contributions (reference values were assigned to all of a structure's bond lengths), V_{θ} denotes bond angle contributions (reference values were assigned to all of a structure's bond angles), V_{φ} represents torsional contribution arising from deviations from optimum dihedral angles, V_{ij} incorporates van der Waals interactions due to non-bonded interatomic distances, V_{hb} symbolizes hydrogen-bond energy function and V_{el} stands for electrostatic energy.

In addition, the total potential energy deviation, ΔE_{total} , was calculated as the difference between total potential energy of the complex system and the sum of the potential energies of isolated individual molecules, as follows:

$$\Delta E_{\text{Total(A/B)}} = E_{\text{Total(A/B)}} - (E_{\text{Total(A)}} + E_{\text{Total(B)}}) \dots\dots\dots(2)$$

The molecular stability can then be estimated by comparing the total potential energies of the isolated and complexed systems. If the total potential energy of complex is smaller than the sum of the potential energies of isolated individual molecules in the same conformation, the complexed form is more stable and its formation is favoured [15].

$$E_{\text{MAA}} = 19.755V_{\Sigma} = 1.901V_b + 9.759V_{\theta} + 2.733V_{\varphi} + 5.362V_{ij} - 0.001V_{hb} \dots\dots\dots(3)$$

$$E_{\text{PEGDMA}} = 13.774V_{\Sigma} = 0.483V_b + 3.813V_{\theta} + 4.793V_{\varphi} + 4.684V_{ij} \dots\dots\dots(4)$$

$$E_{\text{PEGDMA-MAA}} = 23.304V_{\Sigma} = 2.151V_b + 12.543V_{\theta} + 9.357V_{\varphi} - 0.733V_{ij} - 0.014V_{hb} \dots\dots\dots(5)$$

$$\Delta E = -10.229\text{kcal/mol}$$



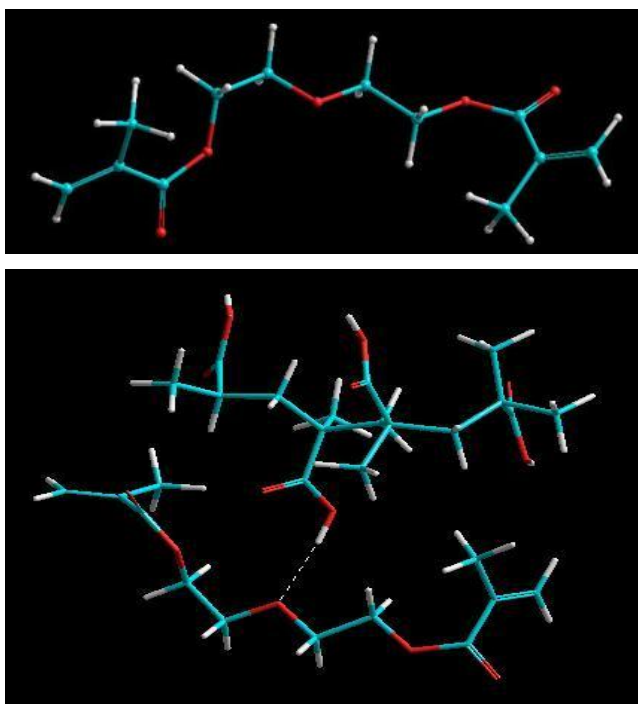


Figure 1. Visualization of geometrical preferences of a) MAA; b) PEGDMA; and c) MAA-PEGDMA along with H-bonding after molecular simulation in vacuum. Color codes: C (cyan), O (red), N (blue) and H (white).

Formation of polymeric assemblies

The PEGDMA was modeled in the form of four monomeric components representing PEG4000, for better efficiency, in terms of computational time and modeling space. The energetic profiles of the formation of the polymeric assemblies viz. PEGDMA-MAA in vacuum, are represented by energy equations 3-5 and the conformational profiles for the same are depicted in Figure 1. The energy equations demonstrated that the polymeric systems were highly stabilized in terms of respective bonding and non-bonding energy factors. The energy stabilization with negative steric energy of -10.229kcal/mol in case of PEGDMA-MAA suggested good compatibility and miscibility. Additionally, the geometric conformation and the hydrogen bonding suggests the involvement of the -COO functionality of MAA with the -COC- functionality of PEGDMA.

Strikingly, the formation of the conjugate introduced torsional deviations from with values in the range of ~4kcal/mol which lead to a destabilized molecular entity. However, the complex was stabilized by generalized energy components in terms of optimum dihedral angles contributions (bonding energies) as well as London dispersion forces and hydrogen bonding (non-bonding interaction).

The van der Waals interactions added most to the stabilization and retrieved high negative values which may be due to PEG-chain acting as filler in the space lattice of the binary system (Figure 2). Furthermore, the H-bonding, though not contributing as much as van der Waals forces, displayed exponentially lowered energies caused due to H-bonds introduced as explained earlier. These non-bonding, from H-bonding to van der Waals forces, may be due to the hydrophobic interactions arising from the inclusion of amphiphilic PEG which further lead to the formation of particles.

Investigation of properties involving micro and nanoparticulate formation and insulin entrapment and release

The presence of the -COOH on the surface of the polymeric assemblies inspired our curiosity for their possible interaction with the incorporated protein - insulin. We therefore, modelled insulin with PEGDMA-MAA nanoparticles in stoichiometric quantities relative to the concentrations used in the preparation of the drug loaded micro and nanoparticles and hence modeled them as Insulin-PEGDMA-MAA for stoichiometric similarity.

$$E_{INS} = -92.617V_{\Sigma} = 1.266V_b + 4.719V_{\theta} + 6.626V_{\phi} + 4.858V_{ij} - 2.642V_{hb} - 107.446V_{el} \dots\dots\dots(6)$$

$$E_{PEGDMA-MAA-INS} = -76.184V_{\Sigma} = 4.583V_b + 30.449V_{\theta} + 21.533V_{\phi} - 17.123V_{ij} - 2.832V_{hb} - 112.795V_{el} \dots\dots\dots(7)$$

$$\Delta E = -6.871\text{kcal/mol}$$

As evidenced from Figure 3, PEGDMA-MAA is highly conjugated with the protein structure as there are -COOH end groups in the polymer matrix to interact with the -NH₂ groups of insulin leading to an energy stabilization of ~7kcal/mol. The H-bonding in addition to the electrostatic stabilization for Insulin-PEGDMA-MAA complex may lead to a polymer-protein-conjugated system further densifying the polymeric matrix which is similar to the finding in our previous publication, that insulin loading efficiency is higher in nanoparticles because of the high acid value on account of surface -COOH groups [10]. We hereby propose that high and rapid release of insulin from PEGDMA-MAA microparticles, in comparison to nanoparticles, may also be due to porous matrix and loose binding of insulin to the microparticle polymeric matrix. The conolly molecular surfaces shown in Figure 3 display the *in silico* shape, morphology and network structure of the fabricated drug-loaded microparticles. It is evident that PEGDMA-MAA developed a well-oriented and regular matrix conformation. This is in corroboration with the SEM morphological analysis studies discussed in previous publication [10].

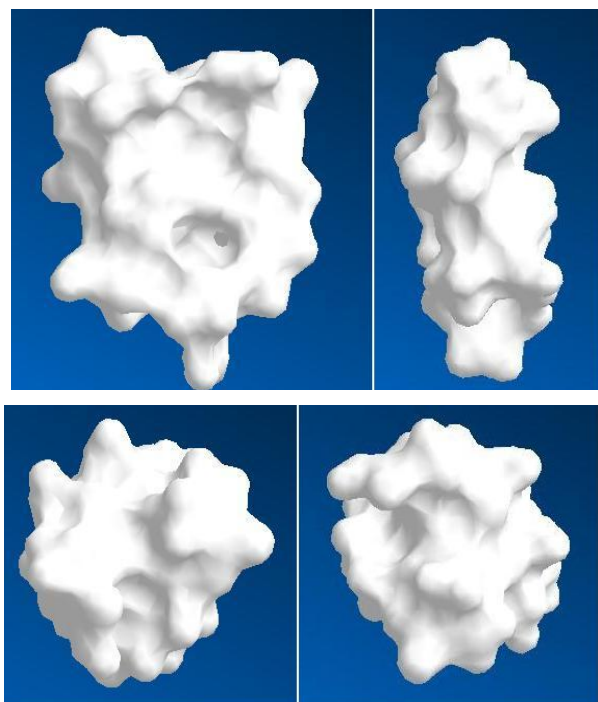


Figure 2. Connolly molecular electrostatic potential surfaces for microparticulate matrix in solvent accessible solid matrix display mode as viewed from various perspective angles.

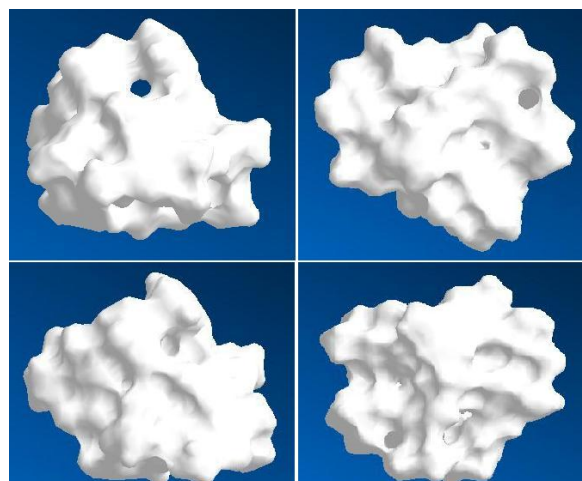
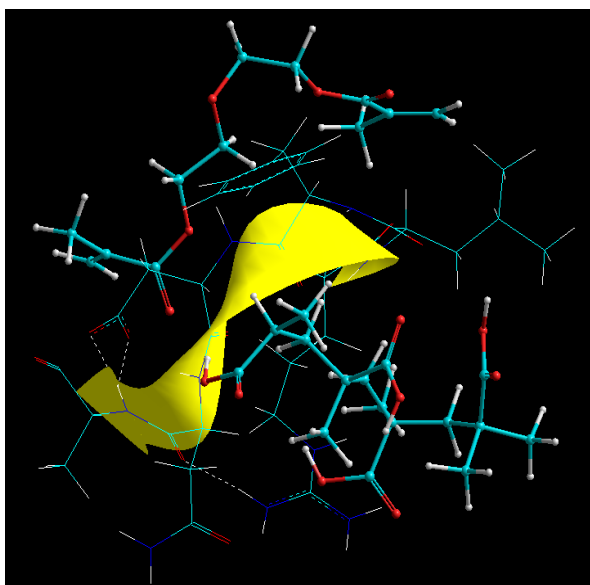
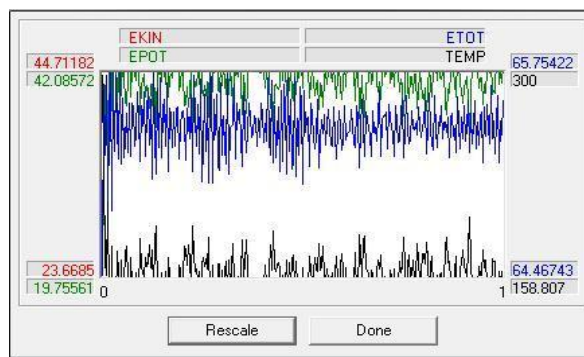


Figure 3. Visualization of geometrical preferences of insulin molecule in complexation with PEGDMA-MAA after molecular simulations in vacuum. The polymer matrix is rendered in ball & stick mode and peptide molecule in stick mode (element color coded) and thin-ribbon secondary structure (yellow). Color codes for elements: C (cyan), O (red), H (white), and P (yellow). The respective Connolly molecular electrostatic potential surfaces for insulin loaded microparticulate matrix in solvent accessible solid matrix display mode are also showcased in various perspective angles.

Elucidation of IPEC stabilization using dynamic simulations

Molecular dynamic simulation was successfully used to further investigate the energy profile of interactions between the functional groups in the PEGDMA and MAA. Figure 4, 5 and 6 represents the main energy attributes inherent to the formation and properties of PEGDMA-MAA.



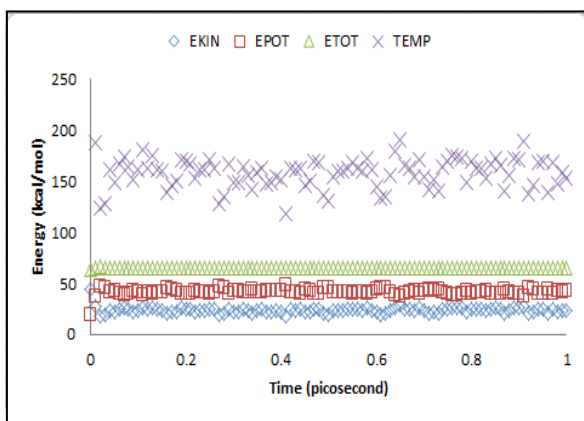


Figure 4. Kinetic energy (EKIN), potential energy (EPOT), total energy (ETOT) (kcal/mol), and temperature (Kelvin) profile for MAA.

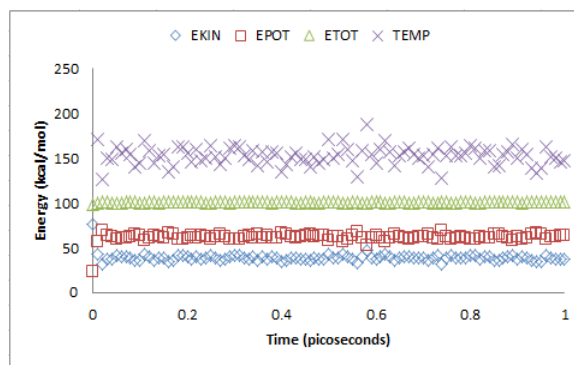
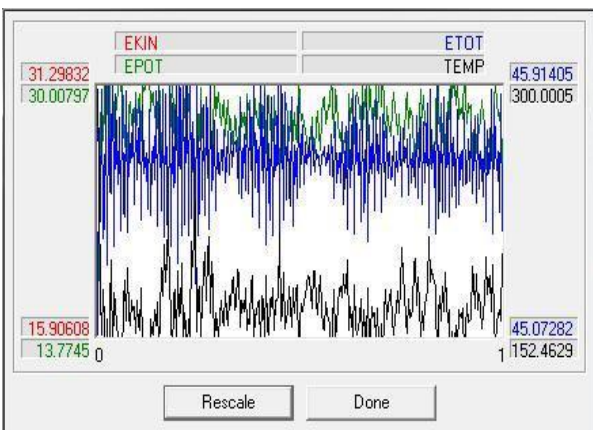
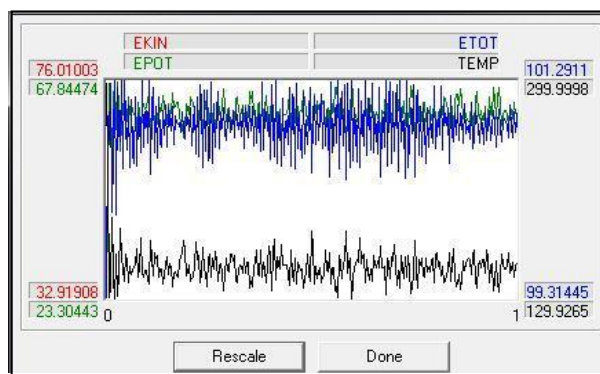


Figure 6. Kinetic energy (EKIN), potential energy (EPOT), total energy (ETOT) (kcal/mol), and temperature (Kelvin) profile for PEGDMA-MAA.

From Figure 4-6, we see that by 1000 time-steps, the potential and kinetic energy were fluctuating about equilibrium values. The atoms vibrate about fixed positions as a result of their thermal energy, and give rise to the graphs in Figure 4-6 depicting typical solid-type behaviour. In case of MAA, EKIN and EPOT displayed a significant difference among the constituent energy values (Figure 4). However, for PEGDMA, EKIN and EPOT displayed equivalent values as evident from the energy overlap in Figure 5. The final ETOT value in case of PEGDMA-MAA was stabilized by ~11kcal/mol further confirming the computational results (formation and conformation) obtained in molecular mechanics simulations. The energy minimization is mainly due to potential energy changes which are evident from the EPOT_{PEGDMA-MAA} displaying a significant flotation owing to the interaction among the atoms of PEGDMA and MAA which is also evident from oscillatory form of EKIN_{PEGDMA-MAA}. Furthermore, the total energy varied in direct proportionality to kinetic energy confirming the presence of a spring-mass system in PEGDMA-MAA which is in good corroboration with the characterization results obtained during experimental investigations. Interestingly, it was found that the potential energy was proportional to the

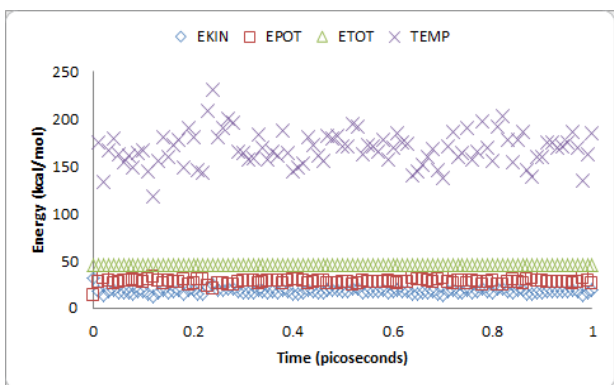


Figure 5. Kinetic energy (EKIN), potential energy (EPOT), total energy (ETOT) (kcal/mol), and temperature (Kelvin) profile for PEGDMA.

increase in the kinetic energy, obeying the well-known behaviour of non-harmonic oscillator.

CONCLUSION

With reference to the results from the vacuum system and protein-polymer simulations, it can be concluded:

- 1) Formation of the PEGDMA 4000:MAA copolymeric particles was favoured by the energy stabilization.
- 2) Copolymeric microparticles are porous in nature which facilitates a better release of insulin from microparticles in comparison to nanoparticles. In our previous publication a higher insulin loading was observed and reported in nanoparticles, compared to the microparticles, but a better insulin release was recorded from microparticles [10]. The probable reason for higher loading of insulin in nanoparticles is the presence of higher carboxyl functionality on the nanoparticles surface to which insulin bind chemically and therefore is not released later in appreciable proportion.

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