

# Novel pH-Responsive Nanohybrid for Simultaneous Delivery of Doxorubicin and Paclitaxel: An In-Silico Insight

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## Research article

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# Abstract

**Background:** The distribution of drugs could not be controlled in the conventional drug delivery system. This has led to the development of a smart nanoparticle-based drug delivery system, known as smart drug delivery systems. In cancer research, novel biocompatible nanocarriers have received much attention for various ranges of anticancer drugs.

In the current study, the effect of a novel co-polymer "dimethyl acrylamide-trimethyl chitosan" was investigated on drug delivery of doxorubicin and paclitaxel utilizing modified fullerene nanocarrier.

This study was performed via molecular dynamics simulation based on acidic pH sensitivity of the cancer microenvironment. Furthermore, hydrogen bonds, diffusion coefficient, gyration radius, and drug-carrier interaction energies were investigated here. Interestingly, a simultaneous pH and temperature-sensitive system was proposed for paclitaxel and doxorubicin for a co-polymer. A pH-sensitive and temperature-sensitive copolymer was used based on trimethyl chitosan and dimethyl acrylamide, respectively. In such a dualistic approach, co-polymer makes an excellent option to have two properties in one bio-polymer.

**Results:** The results suggest the dramatic and indisputable role of the co-polymer in the release of doxorubicin and paclitaxel in cancerous tissues, as well as an increased biocompatibility and drug uptake in a neutral state. The validation test was performed by repeated simulations of a similar article. The results are very close to those of the reference paper .

**Conclusions:** Overall, conjugated fullerene and dimethyl acrylamide-trimethyl chitosan (DMAA-TMC) as carriers can be a good proposition for loading, delivery, and release of anti-cancer drugs based on a pH/temperature-sensitive smart drug delivery system.

## Introduction

Multiple factors are involved in cancer initiation and progression (1). These factors can be intrinsic, such as susceptibility to Chronic myelogenous leukemia (CML) in a newborn with Philadelphia Chromosome or extrinsic, including tobacco smoking, diet, infectious disease, ion and non-ion radiation, and so on. Through complex mechanisms, these factors will promote the expression of oncogenes (e.g. MDM2 and BCL) or suppresses the tumor inhibitor genes (genome guardians) such as P-53 and RB (2–9). Nowadays, various treatment methods including chemotherapy, radiotherapy, and target-therapy are common ways of cancer therapy, each of which has specific pros and cons. Researchers are focusing on optimizing the drug's efficacy while reducing undesired side effects (10). In the current century, nanobiotechnology has gained ground in cancer treatment and diagnosis (11, 12). Development in targeted nano-drug delivery has resulted in the progression of the smart drug delivery concept (13). The maximum level of optimization, a surge in the therapeutic index and drug concentration in affected tissue, and reduced damage to healthy cells will be achieved in such a system (14, 15).

Doxorubicin (DOX) and Paclitaxel (PAX) are two crucial common combination anticancer agents in cancer therapy. Previous Simultaneous administration studies suggested that DOX/PAX combination could be effective in metastatic cancer, especially in breast cancer. Due to synergism, this combination is also effective in inhibition of multidrug resistance (MDR) cancer cells. DOX, as an anthracycline, interferes with the production of proteins that are essential for cancer cell proliferation and impede cancer growth (16–18). It seems that this drug acts via binding to DNA and subsequent inhibition of nucleic acid production by disrupting the molecular structure and further steric hindrance (19). As with any anticancer drug, one of the undesirable side effects can be damaging surrounding cells of the tumor (20). As mentioned above, targeted drug delivery can minimize the impacts of this drug on non-cancerous cells of the body and maximize the drug efficiency for cancer treatment (17, 21, 22). PAX is a novel antimicrotubular agent that inhibits the production of microtubules from tubulin dimers and stabilizes the microtubules by preventing depolymerization (22, 23). This stabilization inhibits the dynamic identification of the microtubule network, which is required for critical interphase and mitotic cellular functions (24, 25). PAX is one of the most effective drugs in the treatment of breast cancer with chemotherapy. Also, utilizing nanocarriers to optimize PAX's efficacy seems to be promising (26).

Nano-based Drug delivery systems have an essential role in the development of chemotherapeutic agents, cancer drug targeting, the selective antiproliferative effect (27), and minimizing adverse drug side-effects (28). Multiple nanoparticles are capable carriers in enhancing the delivery of DOX/PAX; these agents are solid lipid nanoparticles (29). Among these, 60-carbon fullerenes are one of the compounds that have recently received much attention in DOX/PAX drug co-delivery is (30). The 60-carbon fullerenes (C60) are about 1 nanometer in diameter, nearly half the width of a DNA helix; it has a small size and spherical shape, enables it to conveniently cross biological membranes and barriers and reach the cell (31). By having extended surfaces area, the surface properties of these particles can be readily modified and functionalized using functional groups and compound. The functionalization of these particles will increase their solubility, biocompatibility, and potential to deliver various materials within the body. These particles can be used as carriers of biological molecules such as protein, DNA, and drugs. Drug compounds can be loaded onto or within these structures (32). Another interesting issue in drug delivery is co-targeting and co-transportation in which two or more compounds are targeted and transported. Many surveys have studied the potential of fullerene (33). The particular physical and chemical features of C60 as a nanocarrier for anticancer agents for drug delivery include size, triangular shape, surface charge, surface chemistry, hydrophobicity, loading's potential, and especially the ability to cross various biological barriers in vivo without inducing an immune response.

C60 can bond with specific cell receptors and intracellular target molecules for targeted delivery of therapeutic agents (34). Research has shown that C60 enter the cell vertically through mediated endocytosis mechanism because of there similarity to clathrin. The drug can be encapsulated by the C60 and protected during circulation through the body. After reaching the target site, the encapsulating materials will be degraded after the drug release from the C60. The encapsulated drug should be proportional to the diameter and size of the C60 (35). Despite their specific inherent properties, relevant

concerns have been mentioned regarding the toxicity of C60, as several studies have manifested that pristine C60 can instigate biological destruction (35).

In this work, C60 bioconjugation with a novel biodegradable and biocompatible polymer dispels its biological concerns and converts it to a safer, more reliable nanocarrier. On the other hand, many investigations indicated that the C60 functionalized by a carboxylic group rendered it pharmacodynamically and pharmacokinetically better for drug delivery. Overall, the pH-sensitive C60 modified with non-bonded interaction of dimethyl acryl amide-trimethyl chitosan (DMAA-TMC) in this work produces a biocompatible C60 that can capably be nominated as smart and target delivery systems. DMAA-TMC, as a modified polysaccharide from chitosan, plays a vital role in adsorption enhancement of novel macromolecule delivery systems. In previous studies, the role of chitosan and acrylamide polymers was clarified separately in the improvement of drug delivery systems. In our current study, the combination of two polymers DMAA and TMC, in silico by C60 carrier was investigated for the first time. Moreover, it's non-bonded interaction with C60 and positive surface charge induce the specific properties in drug adsorption and drug release. Because of pores in DMAA-TMC, modified C60 was evaluated as a potential tool to improve the loading and co-release of DOX/PAX in physiological and cancerous pH, respectively (36).

Molecular dynamics is a powerful tool that can provide qualitative and quantitative information on the physicochemical interactions and mechanisms of pharmaceutical systems. In the study of molecular dynamics, a system is first considered that consists of "N" particles inside a box called "simulation box". The location and velocity of the particles at each step can be used to calculate all the static and dynamic properties of the system. From the theorists' point of view, the importance of molecular dynamics studies is that they provides accurate quasi-experimental results for a well-defined model. Molecular dynamics serves somewhere between experiments and theory and is considered more attractive than machine learning simulations(37, 38) .

Empirical experiments are highly important but may impose a significant financial burden on researchers. Hence, there are many studies today on molecular dynamics to simulate drug delivery systems in cancer. However, therefore, the C60s as an attractive carrier for drug release were used to compare the uptake, diffusion, and release of DOX and PAX from C60 in the presence of chitosan polymer. Given the unique properties of C60, this could be an excellent introduction to the wider use of carbon C60 in the loading and release of anticancer drugs (39, 40).

## Method

### Molecular dynamics simulation

Gromacs 5.1.2 software was used to perform the simulation; the input structures were prepared with the OPLS-aa force field. Using ACPYPE script, the parameters of the molecule that were converted to Gromax format. All the particles were placed inside the box, and the tip3p water model was used as the solvent

(41). Energy reduction was performed on all 50,000-step simulation systems by the steepest descent method to eliminate van der Waals interactions and to form hydrogen bonds between water molecules and other molecules. In the next step, the system temperature gradually increased from 0 to 310 K for 100 picoseconds in constant volume, using the Nose-Hoover algorithm (42).

Moreover, the temperature system coupling rate of 0.5 ps used and then at the constant pressure was equilibrated for 200 ps. The Parrinello-Rahman algorithm was used to balance the system pressure. Molecular dynamics simulation was performed at 37 ° C for 50 ns. The cut-off distance was set at 1.2 Particle mesh Ewald (43) was used to calculate the electrostatic force. The linear constraint solver algorithm was used to maintain the length of all links. In order to increase computational speed, the SHAKE algorithm was used to limit the hydrogen atom's bonds (44).

## Carbon nanostructures parameters

The carbon atom charge in these nanostructures was assumed based on the use of naphthalene structure in the zero opslaa force field. The types of bonds between carbon atoms were defined based on amino acids phenylalanine (45), tyrosine (46), and tryptophan (Trp) (47). The angle type is also determined based on the angles of the aromatic amino acid phenylalanine ring. The charge and the parameters of the functional groups on this nanostructure were defined using a similar structure existing in the opslaa force field. Lenard-Jones models and Colombian potentials were used to calculate non-bonding interactions such as electrostatic and van der Waals, respectively.

## Results And Discussion

To predict the co-adsorption of DOX/PTX on the DMAA-TMC functionalized C60, utilizing various computer-based mechanisms, co-release, and aggregation of drugs were investigated in cancerous and physiological conditions with pHs 5.5 and 7.4, respectively.

### *Drug-C60 Aggregation*

Gyration radius ( $R_g$ ) is a factor that enables us to analyze the aggregation and stability of molecules such as polymers and resizing of biological macromolecules such as proteins over time (47). The average of the gyration radius at initial and final time is shown in Table 1 and 2. As shown in Table 1, the gyration radius indicates the accumulation of molecules in one region. The low  $R_g$  indicated a high accumulation in the location. The Gyration radius of DOX and PAX is about 3 nm, indicating the aggregation radius of drug accumulation on the C60 surface. Due to the dimensions of C60 and simulation boxes, a useful aggregation of drugs is formed around the C60. This revealed that the polymer molecules are clustered together in this simulation. PAX also has a lower radius than DOX, indicating a better accumulation of DOX in comparison to PAX. Complexation due to the accumulation of PAX molecules is more stable and concentrated. The interaction of hydrophilic polymer DMAA with water molecules and C60 helps to coat the nanocarrier better in bloodstream, which can improve the

hydrophilicity of PAX. According to Table 1 and 2 the accumulation of the same drugs is similar in two different pHs states at initial time.

On the other hand, the higher the gyration radius, the greater dispersion between the particles. As shown in Table 2, At acidic pH the Rg increase, the stability and aggregation of systems decrease, and the system disassembled. Hence, the release of drugs facilitated at acidic environment

**Table 1:** Neutral pH

Structure	Doxorubicin	Paclitaxel	DMAA-TMC
Initial gyration radius	3.34 nm	3.46 nm	3.61 nm
Final gyration radius	2.08 nm	2.43 nm	2.67 nm

**Table 2:** Acidic pH

Structure	Doxorubicin	Paclitaxel	DMAA-TMC
Initial gyration radius	3.46 nm	3.57 nm	3.72 nm
Final gyration radius	3.74 nm	3.69 nm	3.91 nm

### ***Drug-nanocarrier interaction***

Hydrogen bonding between two atoms is defined as a donor-acceptor pair with an angle between them less than 30 degrees. The Fig.1 show changes in the numbers of hydrogen bonds over time between polymer-polymer and polymer-drug and C60-drug for all three simulations are shown in Fig.1. Hydrogen bonding can serve as a hydrophilicity indicator of the carrier. Besides, hydrogen bonding is part of the interatomic forces that can contribute to carrier strength and stability. The analysis of the diagrams below shows that DOX is not bounded to C60s but has many hydrogen bonds with DMAA-TMC. This chart illustrates the crucial role of DMAA-TMC in this drug delivery system. DMAA-TMC interacts and bonds with C60, which makes it more hydrophilic. C60s and DOX, as well as PAX, are hydrophobic compounds, and this is a significant drawback for drug carriers; Because hydrophobic compounds aggregate in water and compose large particles disrupting drug delivery and block the bloodstream.

Furthermore, DMAA-TMC has solved this problem by hydrophilic the complex. The graphs also show that PAX did not form hydrogen bonds with C60s, while PAX makes many hydrogen bonds with DMAA-TMC, which is an essential factor in drug delivery. A comparison between PAX and DOX Shows that the DOX-DMAA-TMC hydrogen bonds are more robust than the PAX-DMAA-TMC hydrogen bonds. Therefore, the addition of DMAA-TMC also contributes to better adsorption of DOX as the hydrogen bonds between DOX and DMAA-TMC are relatively stable.

## ***Energy interaction of drug and nanocarrier***

### ***a. Neutral state; pH=7.4***

In the Fig.2 and Fig.3, the interaction of the DOX molecule with C60 is investigated. At neutral pH, electrostatic energy plays a significant part in the total interaction energy. While in the acidic state, the electrostatic energy is zeroed, and the van der Waals energy has a considerable share of the total interaction energy. That is due to the surface charge of the carboxyl functional groups at the C60 surface. The carboxyl group has a negative charge at neutral pH and no charge at acidic pH.

On the other hand, DOX has a positive charge at neutral pH and acidic pH. As a result, in the neutral state, drug, and C60 functional groups, have anonymous and robust electrostatic interactions. The higher the electrostatic energy at neutral pH, the higher the adsorption of the drug onto the nanocarrier surface at this pH. The critical point is that the drug at a neutral pH, which is the pH of the blood, can transfer satisfactorily to the surface of the C60. and C60, by having a strong attraction to DOX, can serve as an excellent carrier for the drug.

Fig.2 shows the interaction between DOX and DMAA-TMC , as can be seen in figure c., the van der Waals interaction is close to zero. Still, there is a significant negative electrostatic interaction between the drug and the polymer. This interaction is due to the positive charge of DOX and the negative charge of the polymer at this pH. The negative electrostatic energy indicates a strong attraction between the drug and the DMAA-TMC. DMAA-TMC is an essential aid in the absorption of the drug.

The part b. in Fig.2 is the energy interaction of PAX with C60. As shown in the figure at neutral pH, the electrostatic energy shows a more significant number, while the energy van der Waals energy is close to zero. C60 is functionalized with carboxyl functional groups. The carboxyl group has a negative charge at neutral pH and no charge at acidic pH. On the other hand, PAX has zero charges at neutral pH. As a result, in the neutral state, the electrostatic energy between PAX and the C60 is close to zero. Van der Waals Energy plays a significant part in the adsorption of PAX onto C60. The following diagram illustrates the energy interaction between PAX and DMAA-TMC at d. As shown in Fig.2, at neutral pH, van der Waals energy shows a more significant number, and electrostatic energy is nearby to zero. PAX has zero charges at neutral pH. As a result, in the neutral state, the electrostatic energy between PAX and DMAA-TMC is close to zero. van der Waals energy plays a significant role in the absorption of PAX onto DMAA-TMC.

### ***b. Cancerous state pH=5.5***

In Fig.4, the interaction energy of the doxorubicin molecule with C60 is investigated. As shown in the figure at acidic pH, the electrostatic energy is shallow and close to zero. At acidic pH, electrostatic energy is zero, and van der Waals energy has a significant contribution to the total interaction energy. This is due to the surface charge of the carboxyl functional groups at the C60 surface. On the other hand, DOX has a positive charge at neutral pH and acidic pH. As a result, in the neutral state, C60 and drug functional groups have nameless charges and find durable electrostatic interactions. Though, at acidic pHs since

C60 and carboxyl group charge becomes zero, the electrostatic interaction energy between C60 and DOX is also zero.

Fig.4.b. Shows the van der Waals and electrostatic energies of PAX and C60. As is clear from the figure, the electrostatic energy is close to zero, and the total energy is approximately equivalent to the van der Waals energy. The zero electrostatic energy is due to the zero charge of the carboxyl group at acidic pH. However, the surface charge of paclitaxel is also close to zero. Therefore, the electrostatic interaction between paclitaxel and C60 is zero, and the van der Waals interaction is fragile. The weak interaction energies lead to a better release of the drug from C60 and are considered a decisive factor for the carrier, which can be very useful in drug release.

Fig.4 c. Shows the interactions between DOX and DMAA-TMC. The interesting point in the below diagram is that the interaction between the drug and the DMAA-TMC in an acidic state has positive electrostatic energy. This means that there is a repulsion between chitosan and the drug, and this is very effective in releasing the drug. Repulsion between DMAA-TMC and the drug causes a better release of the drug from the surface of the DMAA-TMC and C60. In fact, besides biocompatibility and hydrophilicity, DMAA-TMC plays a significant role in the mechanism of drug release in cancer tissue.

The figure 4 shows the interaction between DOX and DMAA-TMC. As can be seen from the figure, the van der Waals interaction is close to zero, but there is a significant negative electrostatic interaction between the drug and the DMAA-TMC. This interaction is due to the positive charge of DOX and the negative charge of DMAA-TMC at this pH. The negative electrostatic energy indicates a strong attraction between the drug and the DMAA-TMC. DMAA-TMC is an essential aid in the absorption of the drug.

Fig.4 d. Shows the interaction energy between PAX and DMAA-TMC in an acidic state. As can be realized from the picture, the electrostatic interaction with van der Waals is zero between the drug and the chitosan, which contributes to drug release. Zero electrostatic energy is due to zero surface charge of PAX.

### ***Drug release mechanism***

As shown at Fig 6. Imine group includes a **double bond** between nitrogen and carbon atoms. The PAX/DOX-loaded nanomaterials via imine linkage could persevere drugs at physiological environment in the system and disassemble at acidic pH through the cleavage of imine bonds, which would face to immediately release DOX/PAX (48).

### ***Validation test***

To validate the computational procedure, a relevant recent article was chosen to reproduce a part of Gibbs free energy ( $\Delta G$ ) calculation (39). This article has studied the co-delivery of DOX/PAX by a nanotube-chitosan carrier. The average value of  $\Delta G$  is -20.75 kcal/mol in the reference work for DOX adsorption on chitosan-nanotube (in acidic pH) . The umbrella sampling simulation in GROMACS software was used to reproduce these data. Simulation results yielded a  $\Delta G$  value of -21.64 kcal/mol that

was near that of the reference study. These measurements can provide proof of this work and show consistency with previous works (39).

## Conclusion

Combining biomaterials with nanoparticles is one of the essential functions of the smart drug delivery system. The results showed that finding new carriers with new compounds could be useful in improving the pharmacokinetic properties of pharmacodynamics and the therapeutic processes of drugs. Due to its significant implication in a variety of cancers, DOX is the focal point of drug research.

PAX is a natural source drug that develops taxel group drugs in the drug field. The molecular study of DOX-based carbonic C60 was the subject of this study, showing that C60 could be a suitable carrier for pH-sensitive smart drug delivery by pH modification mechanism. In this computational work, the interactions between DOX and PAX with C60 were investigated at acidic and neutral pHs, along with the study of drug absorption and release at neutral and acidic pHs, respectively. The exciting results show that the drug is highly absorbed in neutral pH (the normal pH of human blood), and its desired release occurs at acidic pH (the normal pH of cancerous tissue).

The presence of a biopolymer can affect the biocompatibility and biodegradability of the nanocarrier. Also, the effect of trimethyl chitosan as a suitable compound was investigated in this study. The results show that the combination of C60s and DMAA-TMCis sufficient for simultaneous absorption and release of DOX and PAX. As a suggestion for future work, this system can be tested both in the laboratory environment and in the tissues of living organisms.

In the physiological state (pH = 7.4), the DMMA is attached to chitosan so that its interaction will result in increased absorption and hydrophilicity. On the other hand, this polymer is segregated from chitosan in the acidic state (pH = 5.5), which improves the release of anticancer drugs. DMAA is a temperature-sensitive biopolymer that is susceptible to degradation in cancerous environments due to increased temperature. Furthermore, DMAA-TMCis a pH-sensitive polymer, thus the dual-sensitivity mechanism demonstrates the high capability of this novel nanocarrier. DMMA, as an amphiphile polymer, has many advantages in drug development, including better nanocarrier aggregation, decreased toxicity, and reduced nanoparticle size for cell penetration.

On the other hand, the electrostatic interactions of DOX and van der Waals interactions of PAX with C60s decrease and the drugs are more easily released in the cancerous environment. Also, the pharmacokinetic problems of PAX can be underestimated due to improved solubility and penetration, as well as reduced cardiac toxicity of DOX owing to its smart release. The previous simulations in similar articles were repeated as a validation test.

## List Of Abbreviations

<b>Abbreviations</b>	<b>Full form</b>
C60	Fullerene
CML	Chronic myelogenous leukemia
DDS	Drug delivery systems
DMAA-TMC	Dimethyl acryl amid- trimethyl chitosan
DOX	Doxorubicin
Fig	Figure
GROMACS	Groningen machine for chemical simulations
<i>OPLS-aa</i>	Optimized Potentials for Liquid Simulations-all atom
PAX	Paclitaxel
Rg	<i>Radius of Gyration</i>

## Declarations

### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### Availability of data and materials

Not applicable

### Competing interests

The authors declare that they have no competing interests

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### Authors' contributions

Reza Maleki; conceptualization, methodology, software, validation

Mohammad Dahri; conceptualization, visualization, data curation

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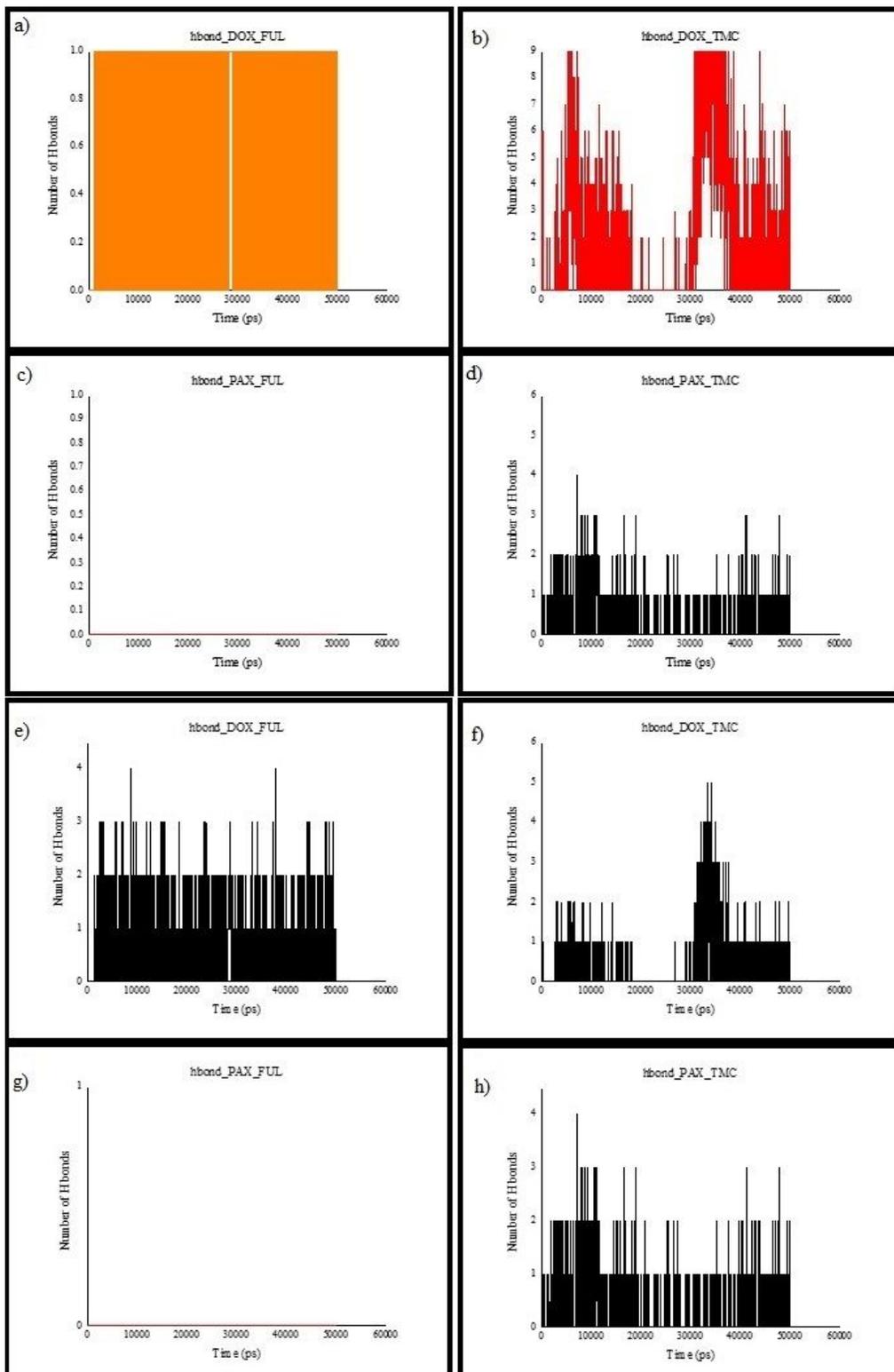
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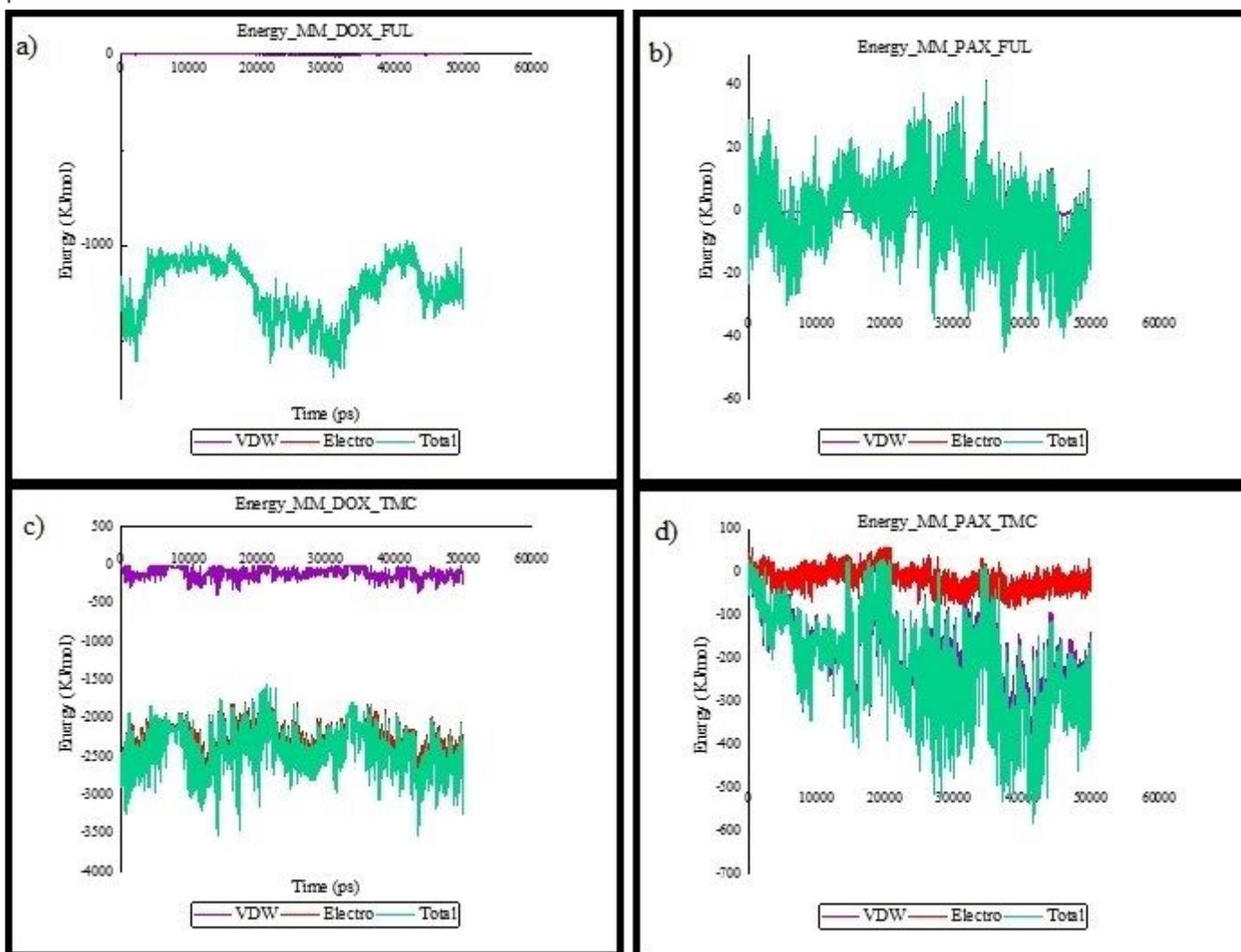
## Figures



**Figure 1**

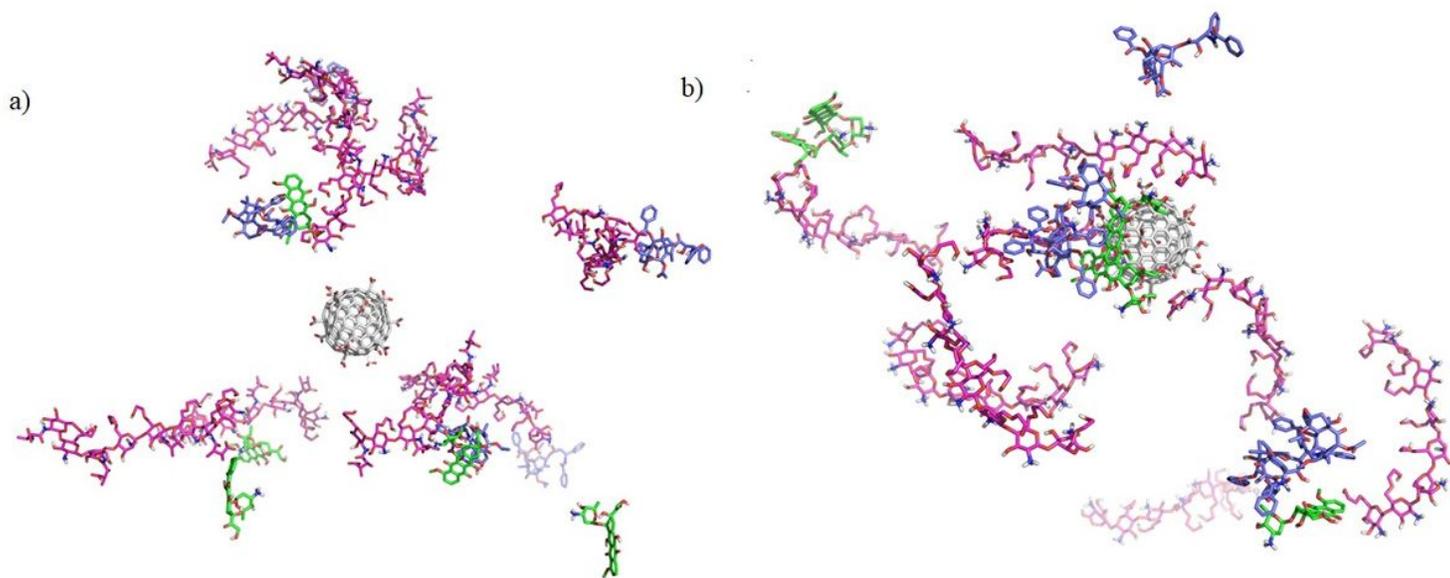
Interaction hydrogen bonds a) The number of hydrogen bonds between DOX and C60 versus time in neutral pH b) The number of hydrogen bonds between DOX and DMAA-TMC versus time in neutral pH c) The number of hydrogen bonds between PAX and C60 versus time in neutral pH d) The number of hydrogen bonds between DMAA-TMC and PAX versus time in neutral pH e) The number of hydrogen bonds between DOX and C60 versus time in acidic pH f) The number of hydrogen bonds between DOX

and DMAA-TMC versus time in acidic pH g) The number of hydrogen bonds between PAX and C60 versus time in acidic pH h) The number of hydrogen bonds between PAX and DMAA-TMC versus time in acidic pH



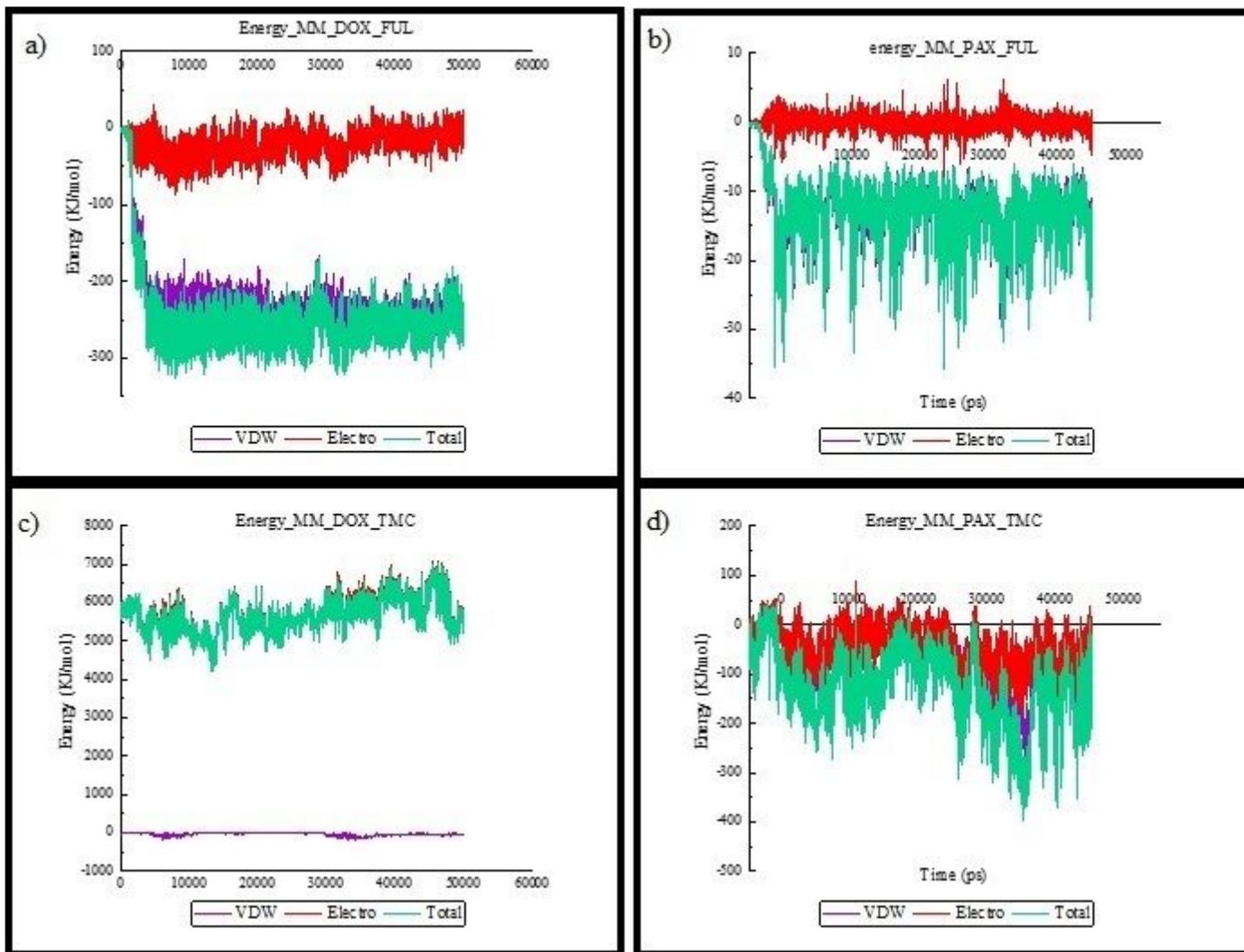
**Figure 2**

Interaction energies between C60 and Drugs, at pH=7.4 a: DOX-C60 b: PAX-C60 c:DOX-TMC d: PAX-TMC



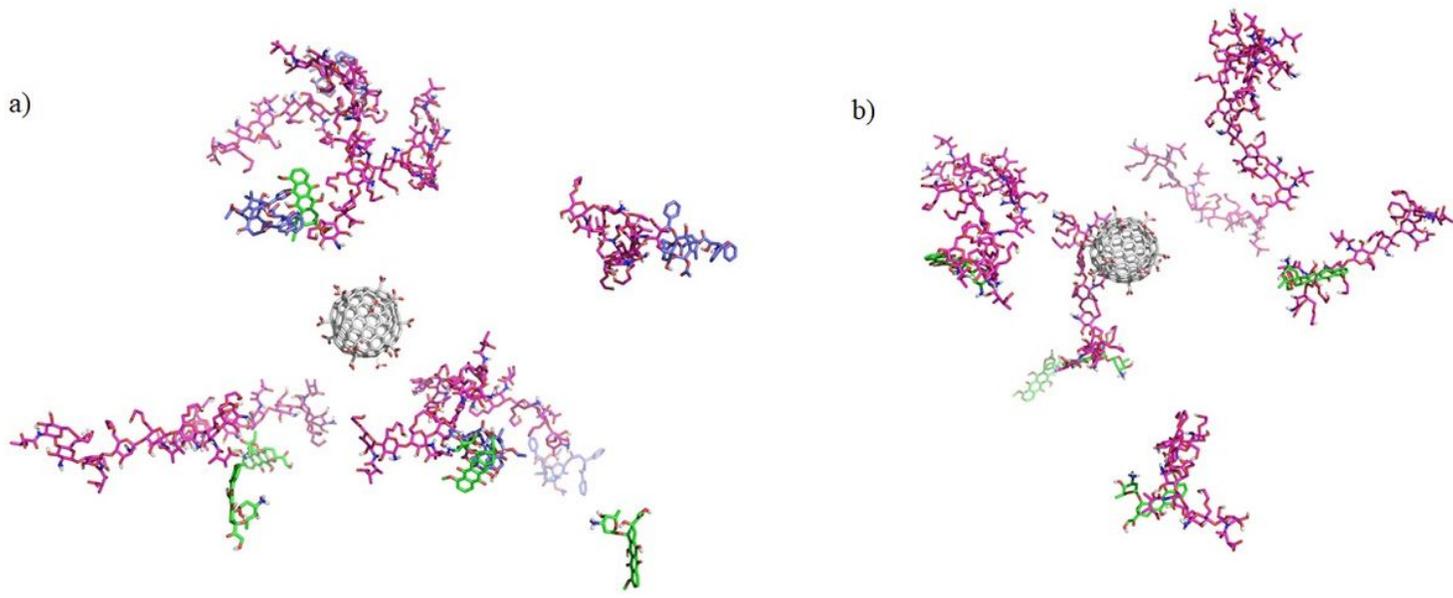
**Figure 3**

Molecular simulation of DMAA-TMC functionalized C60; a: t=0 ns, b: t=50 ns at pH=7.4



**Figure 4**

Interaction energies between C60 and Drugs, at pH=5.5 a: DOX-C60 b: PAX-C60 c:DOX-TMC d: PAX-TMC



**Figure 5**

Molecular simulation of DMAA-TMC functionalized C60; a: loaded, b: unloaded with PAX at pH=5.5

## Dimethylacrylamide-Trimethyl chitosan (DMAA-TCM)

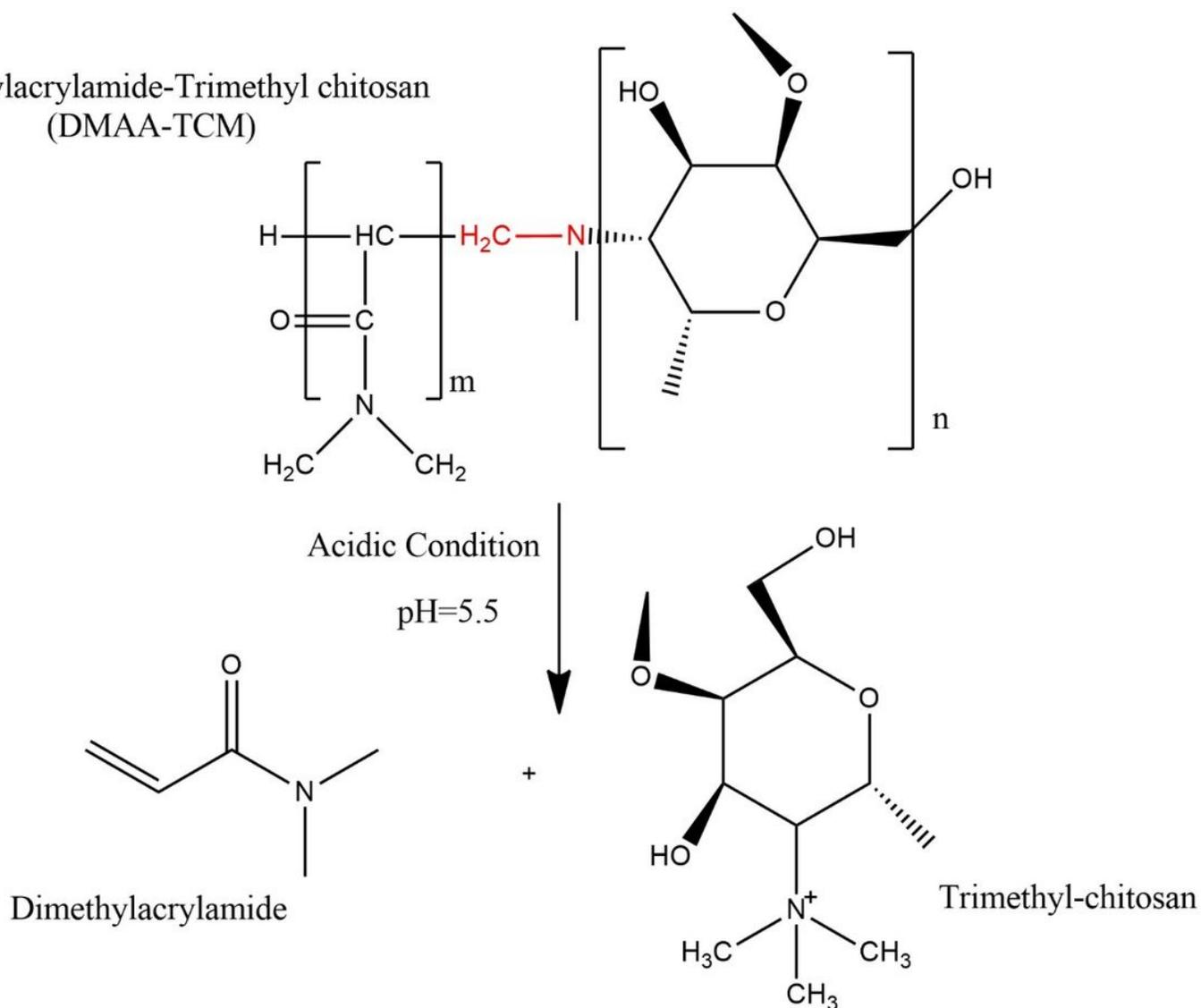


Figure 6

DMAA and TMC dissamlation mechanism at Acidic pH

## Supplementary Files

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- [graphicalabstract.jpeg](#)