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Knowledge-driven prediction and protective encapsulation of small molecules and phytochemicals

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Abstract
Phytochemicals such as 'Caffeine' act as mood stimulants and has become part of daily routine for many people. It is also part of many drugs to cause therapeutic effects and has been used as model drug to study controlled and targeted release in intestine. We checked the feasibility of using *in silico* molecular docking analysis as a screening step to choose the most suitable combination for encapsulation of caffeine-monomer. We also prepared microspheres with the predicted monomers and checked yield and drug release profile.

To test a screening approach for decision of a suitable Caffeine-monomer combination, we performed *in silico* rigid body molecular docking between Caffeine and 10 monomers using Hex™ software. The best docked structures were energy minimized and compared for their stability. Caffeine loaded Chitosan-TPP polymer combinations were made using ionotropic-gelation method with different concentrations of Chitosan, TPP and Caffeine. The encapsulation time was also varied and all combinations were checked for drug encapsulation efficiency and drug release profile.

Molecular docking studies revealed that Chitosan-Caffeine combination has the most stable conformation (lowest energy) among natural polymers. Caffeine-loaded Chitosan-TPP microspheres made with 2% TPP, pH 7.5, 1 % Chitosan, 0.4 % Caffeine and 20 mins stirring time were found to be best in terms of high encapsulation efficiency (83%) and Caffeine release profile. They also showed no covalent interactions between Caffeine and Chitosan and perfect spherical surface of resultant microspheres.

In silico molecular docking analysis predicted that analysis drug-monomer complex can be used as a screening tool to choose appropriate final drug-polymer combinations. Chitosan-TPP microspheres emerged as an ideal system for controlled delivery of model drug Caffeine which was confirmed by experimental findings. Our study would be helpful in improving design of controlled release formulations for various small molecule drugs in natural biopolymers.

Keywords: caffeine, drug encapsulation, controlled release formulations, *In Silico* molecular docking

Introduction

Small bioactive molecules in food items such as 'Caffeine' have been taken up since long as part of many beverages [1]. These are mood stimulants and routinely consumed by many people across the world mainly through coffee seeds which contain 1.25 - 2.5% of caffeine. The mode of consumption includes both at hot and cold forms and this has been not been found to affect the activity of this molecule. But upon passage through gastrointestinal tract, it is exposed to harsh conditions such as low pH and digestive enzymes which can indirectly affect its efficacy and may be deleterious to health [2].

Drugs of plant, animal or synthetic origin are the mainstays of modern medicine and India has huge disease burden including diabetes and heart diseases [3]. These drugs have been administered using traditional delivery systems such as injections, tablets, capsules, etc. But, these delivery systems release the drug very quickly into the blood thereby causing rapid fluctuations in the levels of drug in the body. This leads to the patient suffering from drug side-effects (during high concentration) and insufficient therapeutic action (during low concentration). Moreover, repeated doses are required to provide therapeutic benefit to the patient[4]. This leads to lower patient compliance, non-adherence to drug regime and undesirable side-effects to the patient. This is especially true for drugs having low bioavailability and slow gastrointestinal absorption. Thus, nowadays, controlled drug release formulations are being developed to allow controlled release of drug for an extended period of time. This allows less number of dosages, improved stability and better patient compliance [5].

These preparations facilitate controlled decay of the polymer at particular sites such as Stomach (Ethyl cellulose) or intestine (Chitosan, Carrageenan) and maintain drug levels within therapeutic range, thus avoiding the long term side effects of the drug. They are prepared using a number of techniques such as ionotropic gelation, precipitation, micro-emulsion,

emulsification solvent diffusion, polyelectrolyte complexing and thermal cross linking [6]. The decay of these polymers can be controlled by use of cross-linkers to give more control on the duration and extent of drug release. Earlier, many formulations have used synthetic polymers such as PVP, PVA, PAA and PEG, but nowadays natural polymers such as Chitosan, Gelatin, Collagen, Alginate, Starch, Pectin and Cellulose derivatives have become popular, due to their high biocompatibility, use of mild chemicals and less immunogenicity. Regardless of being synthetic or natural, the criteria for selection of encapsulation material has been mostly empirical and as the drug encapsulation and release profile depends upon both the drug and drug-polymer interactions, a preliminary screening must be done before final preparation [7]. Some researchers have used response surface methodology and mice studies for optimization of product preparation [8] and some have utilized functional criteria to access the effectiveness of optimization [9], but every situation demanding optimization requires application of suitable tools.

Advancements in molecular biology, structural biology and computational chemistry has led to refined molecular modelling and simulations of chemical structures and their interactions *in silico*. Molecular docking capabilities has further improved understanding of ligand-receptor interactions. These *in silico* tools have been mostly utilized to screen drug targets out of many potential candidates. Only a few *in silico* studies have been done so far to select the most suitable polymer for the drug to be encapsulated [10]. We proposed that molecular docking could be used to screen polymers for any possible interactions and predict appropriate combination for usage in controlled drug release. A careful preliminary screen of polymers for non-reactivity with a particular drug would increase the chances of successful encapsulation, saving time and work-flow optimization. Thus, we have used *in silico* rigid shape docking between the Caffeine (taken as model drug) and various polymers to select the most suitable polymer for our study. The performance of Caffeine loaded Chitosan-TPP microspheres was taken as a model system for which, its surface characteristics and drug-polymer interactions were estimated based on shape of microspheres, drug encapsulation efficiency and drug release profile.

Material and Methods

Chemicals and reagents

Caffeine anhydrous, Chitosan ($C_{12}H_{24}N_2O_9$), Glacial Acetic Acid and Sodium tripolyphosphate (TPP) were ordered from SRL (India) and other chemicals were procured from Merck (India), unless otherwise stated. The caffeine was dissolved just before use in double distilled water and kept at 5ºC till use. Chitosan was dissolved in 0.1% Glacial Acetic Acid by keeping at 37ºC for 1 hour to avoid any bubble formation. TPP was dissolved in double distilled water and kept at room temperature until use.

Structures of ligand and monomers

The 3-D structures for the ligand Caffeine (3D_CID_2519) and encapsulation monomers, both chemical monomers viz. Ethylene Glycol (3D_CID_174), Carboxymethyl Cellulose (3D_CID_24748), Glutaraldehyde (3D_CID_3485), Glyoxal (3D_CID_7860), Melamine Formaldehyde (3D_CID_93374) and natural monomers viz. beta Carrageenan (3D_CID_102199626), Chitosan monomer or D-Glucoseamine (3D_CID_54026824), Gum monomer or 4-Methylumbelliferyl chitobiose (3D_CID_11970218), Microcrystalline Cellulose (3D_CID_62698) from Pubchem public database [11] were downloaded and saved as '.sdf' file. The molecule structures were checked for presence of any water molecule and removed if any found present.

Molecular docking of ligand and receptors

The ligand and receptor were loaded on Hex 8.0.0 software [12] and positioned for proper docking. Docking control for rigid body molecular docking was set at following parameters: FFT mode: 3D; Sampling method: Range Angles; Solutions: 100; Steric scan: 18. It was then activated keeping all other parameters as default. The docking steps were allowed to proceed until completion of FFT search, final similarity search and finishing. The docked structures were then saved as docked complexes and energies of top 5 docked structures were noted down. The docked complexes were visualized[13] and images were generated using YASARA view software.

Preparation of Microspheres

The Chitosan-TPP microspheres were prepared using a modified ionic cross-linking method [14] with some modifications. Different combinations of Chitosan solution (0.5% w/v, 1% w/v, 1.5% w/v concentration) and Caffeine (at 0.1 - 0.5 % w/v concentration) were mixed together and microspheres were formed by dropping this homogenous solution using a syringe needle into a mildly agitated (rpm < 200) TPP (0.5% - 2% w/v concentration) solution and kept for either 20, 30, 40 or 60 minutes. The prepared microspheres were separated intermittently, washed with distilled water and air-dried for about 24 hrs and kept later for oven drying at 37°C for 3 hrs to remove inherent moisture.

Percentage yield of microspheres

After drying, the Chitosan-TPP microspheres were checked for their weight and percentage yield was calculated by using the total amount of non-volatile components used in the procedure, as:

Percentage yield (w/w) = (weight of dry microspheres/weight of Chitosan + weight of TPP) x 100

Chitosan-TPP microspheres of different sizes (900-1500µm) were prepared and the size of microspheres was observed under Stereomicroscope (Olympus B51, Japan).

Encapsulation efficiency of microspheres

The Chitosan-TPP microspheres were crushed in a mortar-pestle and 50 mg of crushed powder was used for estimating the amount of caffeine present in microspheres. The powder was kept for digestion in 10 ml of 0.1 M HCl for 12 hours at 37ºC in a shaking incubator. Then the solution was filtered using filter paper (Whatman No. 4) and the solution was checked for concentration of caffeine by taking its absorbance at 274 nm [15] using a spectrophotometer (Hitaichi, Japan).

The encapsulation efficiency was calculated using the following relation [16]:

Percentage Encapsulation efficiency = (Actual entrapment level/Theoretical entrapment level) x 100

Rate of *In vitro* drug release

200 mg of the Chitosan-TPP microspheres from different batches was taken to estimate the drug release from the microspheres in a simulated gastrointestinal environment [16]. The caffeine loaded Chitosan-TPP microspheres were dispensed in 150 ml of pH 1.2 buffer in vessel of dissolution apparatus for 4 hours followed by buffer change by

dispensing in 150 ml of pH 6.8 buffer for another 20 hours. 4 ml each of the dispensing solution was taken out to estimate the drug released after 1, 1.5, 2, 4, 6, 8, 12 and 24 hour using spectrophotometry at 274 nm wavelength. The flask was simultaneously replaced with same amount of buffer solution, each time to maintain total volume. The cumulative drug release from the microspheres was determined by comparing each reading with the drug actually entrapped.

Results and Discussion

Molecular docking and choice of monomers

In silico rigid body molecular docking showed stable binding modes with all combinations between caffeine and monomers of encapsulation polymers. The Caffeine-Chitosan polymer was found to have the most stable conformation (lower energy and correct binding mode) among natural polymers while the chemical monomers showed lower energies/binding modes upon their combination with Caffeine as observed for Ethylene Glycol (-62 KJ/mol), Carboxymethyl Cellulose (-105 KJ/mol), Glutaraldehyde (-95 KJ/mol), Glyoxal (-83 KJ/mol), Melamine Formaldehyde (-125 KJ/mol) and natural monomers viz. beta Carrageenan (-134 KJ/mol), Chitosan monomer or D-Glucoseamine (-107 KJ/mol), Gum monomer or 4-Methylumbelliferyl chitobiose (-170 KJ/mol) and Microcrystalline Cellulose (-138 KJ/mol) respectively (Figure 1).

As a thumb rule, higher the binding energy, lower is the stability. In our *in silico* trials, all synthetic polymers showed higher binding energy as compared to natural ones, showing more stability of natural polymers for encapsulation. Further, among natural polymers, the binding energy was lowest for Caffeine-Gum combination followed by Cellulose, Carrageenan and Chitosan. But, the binding mode between Caffeine and Chitosan was found to be most desirable as it showed no steric hindrance. Based on good binding mode (absence of covalent bonds) and low energy between Caffeine and Chitosan, we chose to proceed with selection of Chitosan-TPP combination for encapsulation trials of Caffeine.

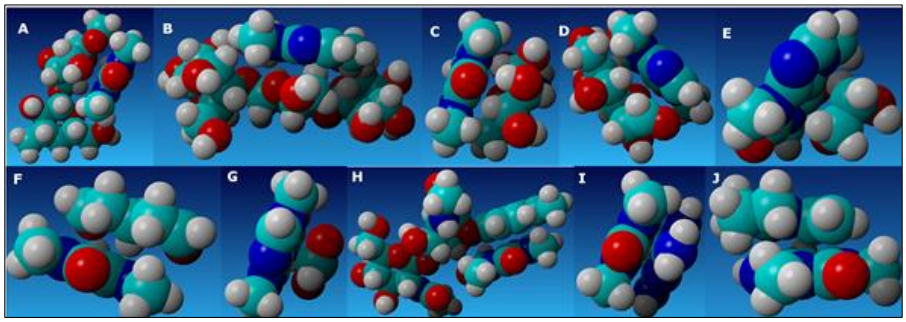

Fig 1: Binding modes of Caffeine for encapsulation with the monomer **A)** Carrageenan **B)** Cellulose **C)** Chitosan **D)** CarboxymethylCellulose **E)** Ethylene Glycol **F)** Glutaraldehyde **G)** Glyoxal **H)** Gum **I)** Melamine Formaldehyde **J)** Vinylpyrrolidinone.

Caffeine-Chitosan-TPP microspheres

Caffeine loaded Chitosan-TPP microspheres were prepared using modified ionic cross-linking method and many batches with the aforementioned combinations were obtained (Table 1). Chitosan-TPP microspheres of different sizes (900-1500µm) were prepared and the size of microspheres was observed under Stereomicroscope (Olympus, Japan). The microspheres had different surface morphology after formation and the batches with 2% TPP, pH 7.5, 1 % Chitosan, 0.2 / 0.3 / 0.4 % Caffeine and 20 / 30 mins stirring time were was found to have desired spherical shape and even surface (Figure 2). The change in concentration of Chitosan, TPP, caffeine and stirring time is known to influence the spherical shape of the microspheres (Singh *et al*., 2015). Spherical shape of microspheres is desirable due to proper drug release from it in each direction and controlled decay. The surface of the microspheres was not found to have any cracks and was smooth for the batch prepared with optimized conditions. We observed that the batch with 2% TPP and 1% Chitosan has little effect of caffeine on the final morphology of the microspheres which was also explained in some of the earlier reviews [17].

Table 1: Caffeine loaded Chitosan-TPP microspheres prepared using the most optimized combinations.

Batch No.	Concentration of TPP solution (%w/v)	Concentration of Chitosan solution (%w/v)	Concentration of drug (%w/v)	Stirring Time (mins.)	Remarks on morphology
11	1.5	0.5	0.2	10	Irregular
21	2	1.0	0.2 / 0.3 / 0.4	20	Spherical
26	2	1.5	0.4	30	Spherical

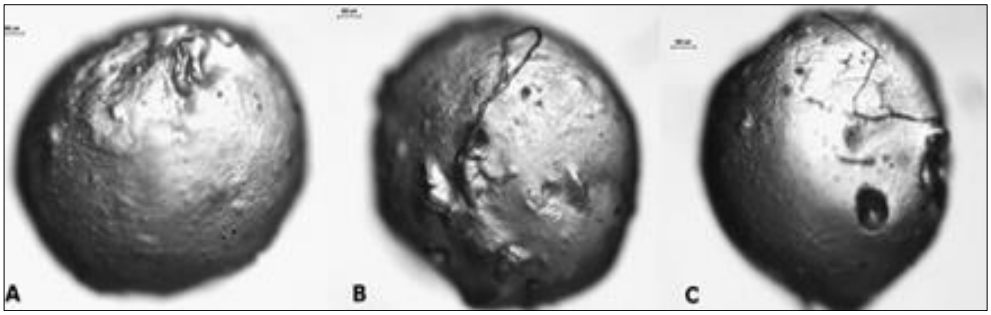

Fig 2: The spherical shape of microspheres obtained using optimized conditions. **A)** Batch 21 **B)** Batch 11 **C)** Batch 26.

Percentage yield of microspheres

After drying and detection by spectrophotometer, the Chitosan-TPP microspheres of different batches were found to contain varying amounts of caffeine inside them (Table 2). The encapsulation efficiency was also found to vary with changing combinations of TPP, Chitosan and Caffeine drug (Table 3). This is indicative of how effective is the polymer-cross linker capacity of encapsulating the drug and the amount of drug actually entrapped inside the microspheres.

Table 2: Percentage yield of microspheres prepared using the most optimized combinations.

Batch No.	Yield (gm)	Percentage Yield (%)
11	0.248	11
21	0.565	25
26	0.339	15

Table 3: Drug release data from the microspheres of most optimized combination (Batch 21).

Sl. No.	Time (hrs.)	% Cumulative Release
1	1	10
2	1.5	15
3	2	20
4	4	35
5	6	43
6	8	57
7	12	80
8	24	95

The batch 21 had extended drug carrying capacity and was observed to show slow release in gastric conditions (low pH) and controlled release during its residence in intestinal conditions (near neutral pH). This is a desirable behavior of a controlled release system for drugs which are susceptible to low pH and their release is sought directly in intestine. This behavior is utilized to ensure bypassing of harsh stomach acids and drug release directly in the intestine [18]. The observed batches were having good yield and the timely release of caffeine from it shows that this polymer-cross linker-caffeine combination could be scaled up for preparation of controlled release formulations.

Conclusions

Drugs have been encapsulated inside protecting polymers for their controlled and extended release. But, the precise decision-making process for choice of drug-polymer combination is empirical and mostly ends up with performing a large number of experimental reactions. We intended to utilize the *in silico* approach to narrow down the number of polymers which need to be tested for appropriate drug encapsulation. We took caffeine as model drug and a total of 10 monomers for this work. *In silico* molecular docking analysis predicted that preliminary analysis of drug-monomer complex can be used as a screening tool to choose appropriate final drug-polymer combinations. Chitosan-TPP microspheres emerged as an ideal system for controlled delivery of model drug Caffeine which was confirmed by experimental findings. Our novel approach would be helpful in improving design of controlled release formulations for various small molecule drugs in natural biopolymers.

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