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COMMUNICATION

Soya Nuggets – A Potential Carrier: Swelling Kinetics and Release of Hydrophobic Drugs

Utkarsh Bhutani, Saptarshi Majumdar*

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In this study, soya nuggets were used as a potential drug delivery vehicle. This work includes the swelling study of soya nuggets followed by the morphology, chemical interactions, mechanical properties and release kinetics. Multiple drugs, both hydrophobic as well as hydrophilic were encapsulated and studied for drug release. This work aims at exploiting soya nuggets as controlled drug release vehicle.

Biodegradable polymers like gelatin¹, chitosan¹, dextran¹, starch¹, alginate², have made ways to some innovative drug delivery vehicles/carriers like thin films³, hydrogels⁴, microgels⁵, micelles⁶, and many more. The idea of these vehicles was to achieve control drug release with minimal side effects⁷. Ensuring the uniform distribution of hydrophobic drugs inside biodegradable polymeric vehicle is a challenging task. To overcome this, synthesis of polymeric beads, polymeric and drug nanoparticles⁸ came into picture together with beta-cyclodextrins⁶. The acceptance of these biodegradable polymeric vehicles is again limited by the use of some toxic chemicals during the formulation stage e.g. cross-linkers like glutaraldehyde⁹. Hydrogels prepared from sodium alginate and gelatine require cross-linkers for improved mechanical properties¹⁰. Other drug delivery vehicles like thin films¹¹, liposomes¹², and peptide hydrogels¹³ have shown potential, but at times they are associated either with burst release of drugs due to improper encapsulation or cost concern. Soya nuggets have been recently reported to give rise to carbon nano-dots¹⁴, used in imaging applications.

This work aims at minimizing the above drawbacks and bring into picture the first use of soya nuggets as potential drug delivery vehicles. Soya is considered as one of the high nutrient protein rich diets. Soya nuggets undergo tremendous swelling which can come handy at the time of drug loading and release. High swelling degree (SD) is a result of high porosity and network structure of swelled soya nuggets.

Soya nuggets were purchased from Nutrela, Ruchi Soya Industries

Department of Chemical Engineering, Indian Institute of Technology, Hyderabad, Yeddumailaram 502205, Hyderabad, INDIA; *Email: saptarshi@iith.ac.in

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Limited, Madhya Pradesh, India. Sodium salt of alginic acid (SA) 'Low Viscosity' (40 - 90 mPas, 1% in water), Piperine (98%) and Phosphate buffer saline - PBS (pH 7.4) were purchased from Alfa Aesar (A Johnson Matthey Company). The detailed experimental methodology is mentioned in the supplementary information.

Soya nuggets due to their high porosity possess a very high swelling degree (SD). The SD was controlled by coating the nuggets with sodium alginate (SA) and cross-linking SA with calcium chloride (CaCl₂). Piperine was used as a model hydrophobic drug. SEM analysis revealed the high porosity of soya, while the FT-IR analysis gave an insight to the stability of the drug inside the nugget. The release of piperine was found to follow Higuchi kinetics. Subsequently, the mechanical properties of swelled soya were evaluated to study the effect of nugget weight on swelling as well as on drug loading. Apart from piperine, metformin, ibuprofen and curcumin were also used for release studies of few other drugs. Dual release of curcumin and piperine was also studied.

Swelling Degree: Burst swelling was observed in pure soya nuggets (non-coated and non-cross-linked). It was 194% in the first 60 min for pH 7.4, while in pH 1.2 it was 199% (**Fig. 1a**). Soya nuggets were observed to give a high degree of swelling in pH 1.2 than pH 7.4 after 24 h. The probable reason behind this was the ionization of amino groups in the essential amino acids at pH 1.2, while as we switch to pH 7.4, it nears the isoelectric point of all the essential amino acid except lysine (**supplementary information (Table 4S)**).

Lower ionization in pH 7.4 leads to less repulsion among the groups resulting in low SD. It is desirable to have low drug release in pH 1.2, which is expected from most of the drug delivery vehicles, i.e. lower release in stomach conditions. But in this case, the behaviour of soya nuggets was found to be just opposite. Hence, soya nuggets were coated with low viscosity (LV) SA.

The choice of SA was influenced by the fact that it is FDA approved biopolymer and remains unionized in pH 1.2, so it could control the swelling rate in pH 1.2. The soya nuggets were coated with 1% SA and studied in pH 1.2 (**Fig. 1b**). However, no improvement was observed. To achieve a control, the samples (SA coated nuggets) were then surface-cross-linked with 1%, 2%, 3% and 5% CaCl₂ (**Fig. 1a and 1b**).

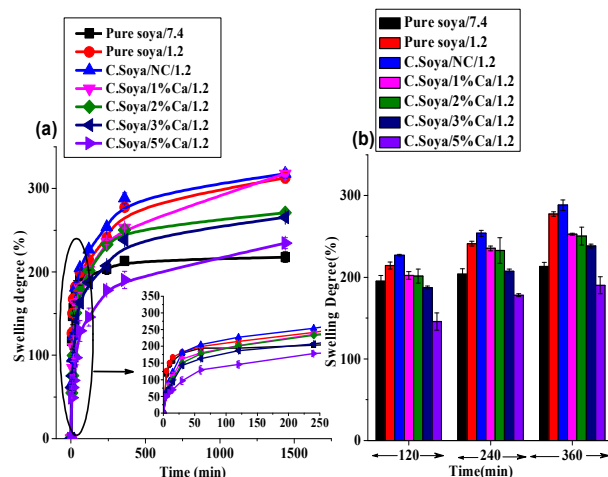


Fig. 1 (a) Swelling degree of soya nuggets (Pure soya, Coated soya (C.Soya) and coated cross-linked) at pH 7.4 and 1.2 (b) Comparison of swelling degree at 120th, 240th, and 360th min.

The coated and cross-linked samples showed a marked decrease in the burst swelling. It decreased from 199% in pure soya (60 min) to finally 129% in 5% CaCl₂ cross-linked samples in pH 1.2 (Fig. 1b). Based on these results 5% CaCl₂ was selected for further testing.

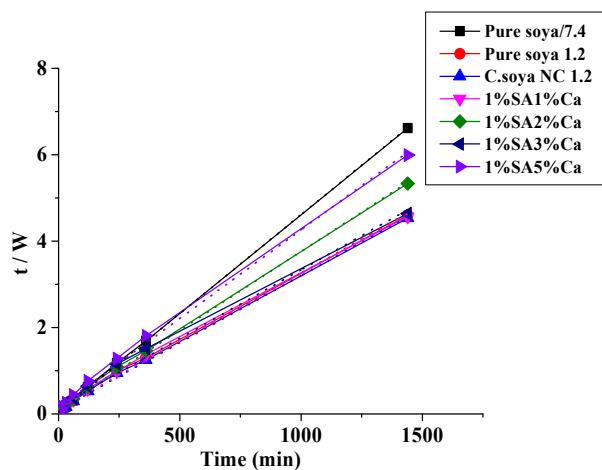


Fig. 2 swelling kinetics: t/W vs time for pure soya, 1% SA coated and CaCl₂ cross-linked samples (1, 2, 3 and 5%) at pH 7.4 and 1.2.

It was further observed that irrespective of the medium used (pH 7.4 or 1.2), the heavier soya swelled less as compared to the low weight counterparts (supplementary information, Fig. 4S). It was then expected that weight of soya should have an effect on the internal structure that in turn caused a variation in SD.

The 24 h swelling kinetics data of soya nuggets was considered to be second order¹⁵ (Fig. 2) (supplementary information Table 1S). After coating and cross-linking there was marked decrease in the initial burst swelling rate at pH 1.2. Diffusion kinetics study was also performed assuming the nuggets to be spherical (supplementary

information Fig. 10S and Table 3S). Solvent diffusion was found to be less Fickian in all the cases¹⁶.

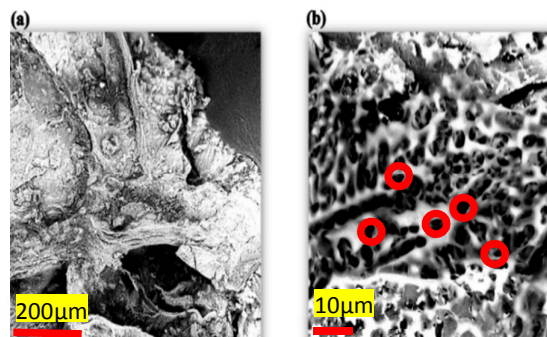


Fig. 3 SEM images of pure/dried soya nugget (a,b)

SEM: The SEM images (Fig. 3a) show highly uneven surface of the nugget with presence of some cracks. These cracks may also contribute to rapid swelling of soya nuggets. The magnified images of soya nuggets showed the presence of numerous pores confirming the high porosity of soya (Fig. 3b).

Mechanical Properties: The motive behind the mechanical analysis was to figure out the effect of nugget weight on its mechanical properties. Four samples were selected based on their weights ranging from 0.9g to 1.5g and were allowed to swell for 24h, which were then evaluated for their mechanical properties. The storage (Fig. 4a) and loss modulus (Fig. 4b) were plotted against strain%. Both increased with weight. Higher values confirmed the stiffness of the material i.e. resistance to deformation/ change its shape. Swelling study has also showed that the higher soya weight has caused lower swelling. Additionally, the storage modulus was greater than the loss modulus, which indicated an elastic behaviour of the soya nuggets.

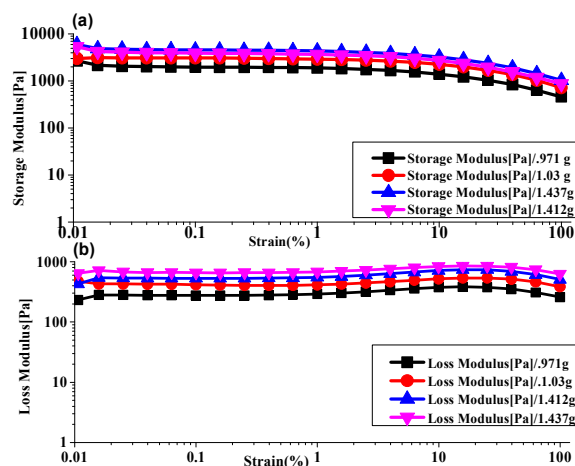


Fig. 4 Storage and loss modulus of pure soya swelled for 24 h in pH 7.4 with increasing nugget weight

FT-IR Analysis: The powdered soya nuggets were studied for FT-IR analysis to determine the functional groups and chemical interactions present in the nuggets. Pure soya nuggets, low viscosity

SA and piperine were evaluated first ((Fig. 5b)¹⁷, (Fig. 5a)¹⁸, (Fig. 5a)). The centres of the broad peaks were considered.

Soya coated with 1% SA was also analysed. The N-H peak in pure soya shifted from 3381 cm⁻¹ to 3403.64 cm⁻¹. The increase in the wavenumber may be due to the interaction of N-H group of pure soya and the carboxylate group of alginate. A slight shift was observed in the N-H bond from primary amine which shifted from 1654.84 cm⁻¹ in pure soya to 1653.38. The reason for this low change could have been the low concentration of alginate used (Fig. 5b)

The interaction peak between N-H group of soya nuggets and carboxylate group of SA further increased to 3431.57 cm⁻¹ due to the interaction of carboxyl groups with the calcium ions (Fig. 5b). The drug stability inside the soya matrix was confirmed by analysing the spectra of pure soya loaded with piperine.

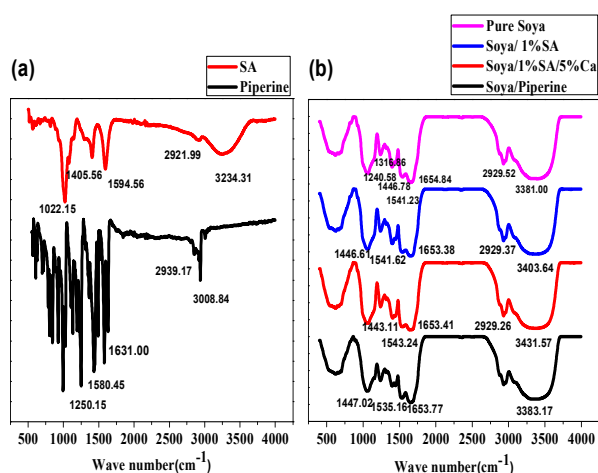


Fig. 5 (a) FT-IR spectra for LV SA and Piperine. (b) FT-IR spectra for pure Soya nugget, soya/1%SA, soya/1%SA/5%CaCl₂ and soya/Piperine.

The peaks observed were very similar to the pure soya sample. The N-H stretch peak shifts from 3381 cm⁻¹ in pure soya to 3383.17 cm⁻¹. The N-O asymmetric peak shifted from 1541.23 cm⁻¹ in pure soya to 1535.16 cm⁻¹ in piperine loaded soya (Fig. 5b). This change could have been due to the interactions between the two oxygen atoms of methylene dioxy ring of piperine with -Nitro groups of soya.

Drug Loading and Release: The loading efficiency (LE, amount of drug encapsulated inside the nugget at the time of drug loading; **Experimental Methodology section in supplementary information**) of piperine was measured and it was observed that the LE varied with the nugget weight. Similar trends were also observed in the porosity of the nuggets. The porosity of nuggets was measured by solvent replacement method¹⁹ (**Experimental Methodology section in supplementary information**) (Fig.6)

The LE (Fig. 7a) and porosity first increased and then showed a decreasing trend with increase in nugget weight. Earlier, the SD and rheology results also confirmed that with increasing weight, the nuggets became rigid and resisted any change in its shape. This was probably the reason for a lower drug loading in high weight nuggets compared to the smaller ones.

Though, the SD of pure nugget was higher in pH 1.2 but the drug release was low in pH 1.2 (Fig. 7b). Hydrophobic nature of the drug may be behind such behaviour.

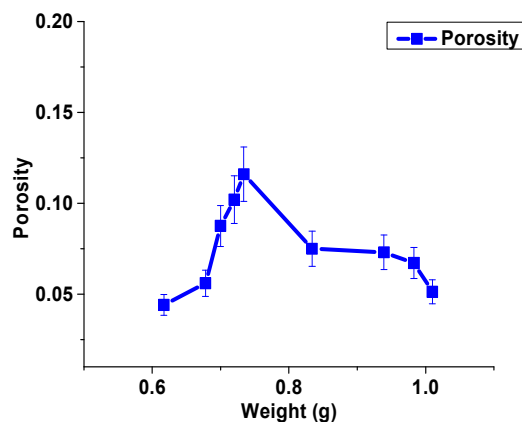


Fig. 6 Porosity of soya using solvent replacement method

The drug release in pH 1.2 observed in the first 240 m was only 33% (Fig. 7c), which was quite promising as within 4-5 h the vehicle moves into the intestine where the maximum absorption of drug occurs. There was a marked decrease in the drug release for 1% SA coated with 5% CaCl₂ cross-linked sample, when compared to the pure nuggets (Fig. 7b and 7c). Few parameters like concentration of SA coating and CaCl₂ were also varied and studied for drug release (**supplementary information, Fig. 6S and 7S**).

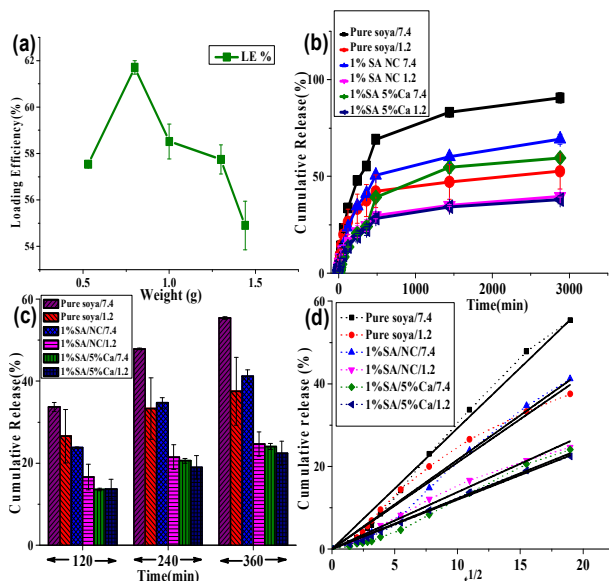


Fig. 7 (a) Loading efficiency of soya nugget with increasing nugget weight (b) Cumulative drug release of piperine from pure soya, 1% SA coated, 1% SA coated and 5% cross-linked soya at pH 7.4 and 1.2 (c) Comparative drug release at 120th, 240th and 360th min (d) Release kinetics: Cumulative release vs t^{1/2}

Since the soya nuggets have a matrix kind of structure (polymer mesh), and were also coated with SA, the release of drugs was

expected to follow Higuchi kinetics²⁰. The kinetic rate constant K_H was obtained for each case and the results were compared (Fig. 7d). The results follow the Higuchi kinetics till 480 min. The value of K_H was the highest for pure soya sample (2.92) (Supplementary information, Table 2S) in pH 7.4. Piperine release in pH 1.2 was slower than pH 7.4 and so was the value of K_H , i.e. 2.15. There was a further decrease in the K_H values for coated and non-cross-linked sample as coating further decreases the diffusion of drug molecules i.e. increase the diffusional resistance. Cross-linking further reduced the K_H when compared to the non-cross-linked samples. (Supplementary information, Fig. 9S and Table 2S) The drug release study was also performed for nuggets of different sizes and can be seen in supplementary information, figure 8S.

Potential of soya nuggets as a drug delivery vehicle was further strengthened when it was tested with other drugs; metformin (hydrophilic drug), curcumin and ibuprofen. Ibuprofen due to its high solubility at pH 7.4 gave a faster release compared to piperine, curcumin and metformin (Fig. 8a). Interesting results were observed when the soya nuggets (1%SA/5% CaCl₂) were tested for dual release of curcumin and piperine. The drug release was performed in PBS with 0.5% tween 20 due to the stability and solubility problems associated with curcumin. A sustained release was observed for 24 h in which piperine has slightly dominated the release (Fig. 8b). This was encouraging as piperine acts as a bio-availability enhancer for curcumin and dual release of those two drugs signifies an important application possibility.

The drug loaded soya nuggets were also stored at 4°C to investigate if the nuggets could sustain their softness and spongy nature for a longer time. The stored nuggets after 30 days were soft and spongy which was promising as they are aimed at achieving a controlled oral drug release. (Supplementary information, Figure. 11S)

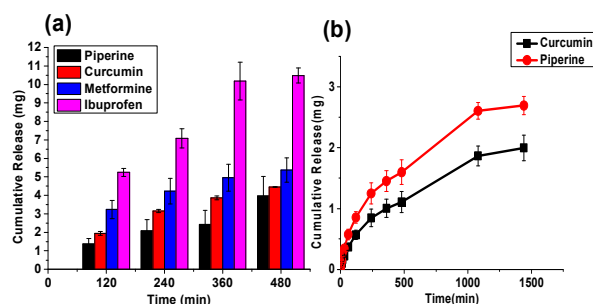


Fig. 8 (a) Cumulative drug release of Piperine, Curcumin, Metformin and Ibuprofen from soya nuggets coated with 1% SA and cross-linked with 5% CaCl₂ (b) Dual Drug release study of Curcumin and piperine in PBS/0.5% Tween 20

Conclusions

The present investigation describes the use of soya nuggets as drug carriers. These nuggets were found to possess high porosity. To control the swelling rate, the nuggets were coated with SA and cross-linked with CaCl₂. The swelling study has confirmed that 5% cross-linked sample swelled at a slower rate in pH 1.2 than pH 7.4. The porosity of the nuggets were tested through SEM and solvent replacement method. The SD was also effected by the nugget weight, which was further confirmed by the mechanical tests performed on swelled soya. The FT-IR analysis confirmed the interactions of sodium alginate-CaCl₂ with the nugget and also

assured the stability of the drug inside. The use of multiple drugs confirmed the potential of soya to encapsulate and maintain the stability of a variety of drugs. The release kinetics was found to be of Higuchi type. Nuggets with 1% SA coating and 5% CaCl₂ resulted in a slow and steady release up-to 24 h. Weight of the nugget also played a crucial role in loading as well as release of drug. The SD of soya nuggets can be taken into advantage, by loading high amount of drug inside. Possibility of having dual drug release was also confirmed in this study. The worldwide acceptability of soya further strengthens its chances to be a potential carrier.

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