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

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Supplementary Information

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Experimental Methodology

Soya nuggets were dipped in SA solution (0.5%, 1%, and 1.5%) for 5 min for coating and then dried again for 24h. The SA coated soya nuggets were cross-linked with (2.5%, 5% and 7.5% w/v $CaCl_2$) by dipping them in $CaCl_2$ solution for 5 min for surface cross linking. Higher cross-linking times were also investigated (15, 30 and 45 min) .The dried nuggets were weighed and immersed in the testing solutions i.e. PBS and 0.1 N HCl. These soya nuggets were taken out at regular time intervals, soaked with tissue paper to remove the surface water and weighed again to get the swelled weight. The SD was evaluated by the following **equation S1**:

$$SD\ \% = \frac{W_s - W_d}{W_d} X\ 100 \quad \text{. (Equation S1)}$$

Where, W_s and W_d represent the weight of swelled and dried soya respectively. The experiments were carried out in triplicate and normalization of weight was done to calculate the final swelling degree. The morphology of the soya nuggets was studied using 'Phenom world ProX SEM'. The samples were coated with gold before the analysis.The analysis for mechanical properties was done using a rheometer (Rheoplus/32 MCR 301, Anton Paar). Parallel plate system (PP75, d=3mm) was used for the analysis. Amplitude sweep test was done with strain varying from 0.01%-100% at constant angular frequency of 10 rad/s. The FT-IR analysis was done to identify the functional groups present in the nugget and also to confirm the stability of the drug inside the nugget. Analysis was performed using Bruker Tensor 37 for a range of 400-4000 cm^{-1}.Soya nuggets swelled more in 0.1N HCl but the hydrophobic drug was not much soluble in HCl. So a solution of HCl/Ethanol was prepared and 20mg of drug was dissolved into the solution. This was used as the loading solution. 25/75, 50/50 and 75/25 HCl/Ethanol solutions were prepared initially and piperine was dissolved in them separately. The drug concentration was measured by UV-VIS spectroscopy (Perkin Elmer Lambda 35) by measuring the absorbance at 340 nm i.e. the λ_{max} for piperine. The loading efficiency (LE) was calculated by the following **equation S2**

$$LE\ (\%) = \frac{Actual\ amount\ of\ drug\ inside\ the\ nugget}{Total\ amount\ of\ drug\ in\ the\ loading\ solution} X\ 100.$$

After 24 h loading, the soya nuggets were dried again for 24 h at 37°C before they were tested for drug release in PBS (pH 7.4) and 0.1N HCl (pH 1.2). The drug loaded nuggets were immersed in PBS (or 0.1N HCl) solution and samples taken at specific time intervals were analysed using UV spectroscopy. Similar weight nuggets were used to minimize the effect of weight on the drug release. The porosity of soya was determined using solvent replacement method. The dried soya nuggets were weighed and immersed in absolute ethanol overnight. The nuggets were weighed again after removing excess ethanol from its surface. The porosity was obtained by using the following **equation S3**

$$Porosity = \frac{M_2 - M_1}{\rho\ V} \quad \text{. (Equation S3)}$$

Where, M_1 and M_2 are the mass of soya nugget before and after immersing it in ethanol, **ρ** is the density of absolute ethanol and V is the volume of the nugget. The bulk density of soya nuggets varies between 0.17-0.22g/cc as per data given by Nutrela, Ruchi Soya Industries Limited, Madhya Pradesh, India.

Physical appearance of Soya nugget

The soya nuggets appear to be dry and having uneven structures **(Fig. 1S (a))**. These pure nuggets were coated with low viscosity (LV) sodium alginate (SA) and were dried again. After drying, the SA coating was clearly visible on the soya nuggets **(Fig. 1S (b))**. Soya nuggets show tremendous swelling and can also be seen in **(Fig. 1S (C))**. It also becomes soft after swelling.

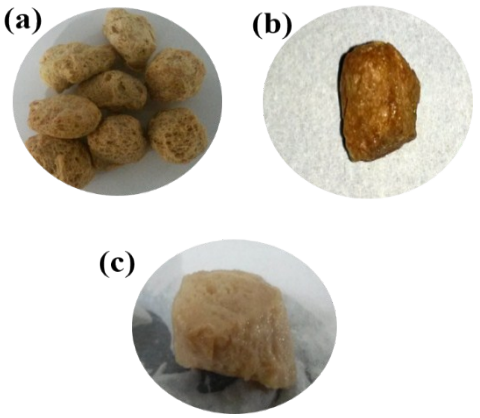

Fig. 1S (a) Pure soya nugget. (b) Soya nugget coated with SA. (c) Soya nugget after 24 h swelling

Swelling Degree (SD): Varying the cross-linking time

The SD of 1% SA coated soya nuggets were studied by varying the cross-linking time. The coated nuggets were cross-linked with 1% $CaCl_2$ solution for 15, 30 and 45 min. It was expected that increasing cross-linking time would result in decrease in SD, but the results followed an opposite trend **(Fig. 2S)**. Probable reason to this could be the presence of free calcium ions penetrating inside the nugget, which increases with higher cross-linking time. Free calcium later invites more water inside the nuggets, which results in higher SD for 45 min cross-linked sample followed by 30 min and 15 min.

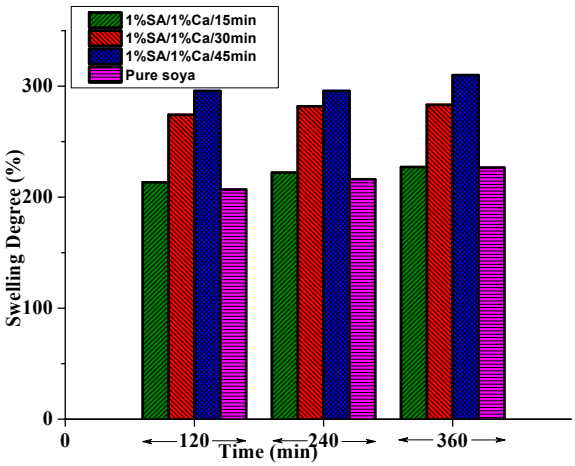

Fig. 2S Swelling degree of 1% SA/1% $CaCl_2$ cross-linked samples with a cross-linking time of 15, 30 and 45 min.

Scanning Electron Microscope (SEM)/Element Detection Software (EDS)

The EDS analysis confirmed the distinct presence of carbon, oxygen, nitrogen and potassium, while phosphorus, calcium, and magnesium were found in traces. Sulphur were also detected during the EDS analysis (sulphur containing amino acids i.e. cysteine and methionine) **(Fig. 3S)**. The high content of C, O and N was obvious due to the presence of carbohydrates and protein, which form the major part of the soya nuggets.

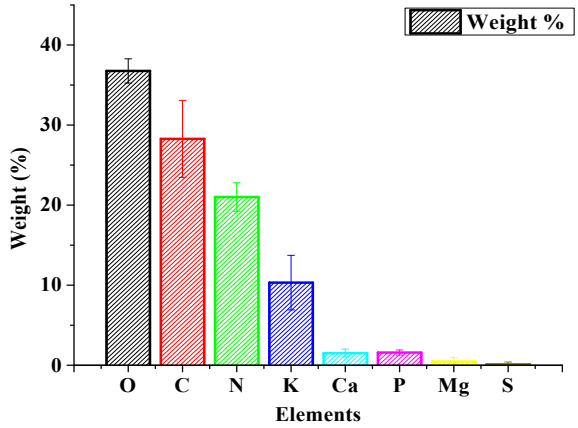

Fig. 3S Elemental analysis of pure soya sample

Swelling Degree: Varying the nugget weight

It was observed that a lower weight nugget swelled more than the higher weight nugget **(Fig. 4S).** To further investigate the reason for this, the swelled nuggets were tested for their mechanical properties and it was found that the storage and loss modulus of the nuggets increased with increasing nugget weight. This was a clear indication that with increasing weight, the structure of the nugget became more rigid and resisted any change in its shape, which led to a reduction in the SD. These results were further supported by the porosity results obtained from solvent replacement method.

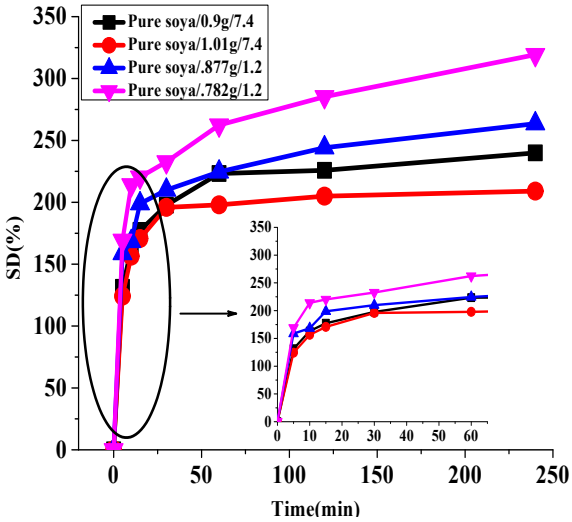

Fig. 4S The effect of soya weight on swelling degree.

Drug Release: Varying the loading solution.

The nuggets swelled more in pH 1.2 than in pH 7.4. So it was expected that a better swelling would give a better loading of drug inside the nugget. Piperine was used as model hydrophobic drug. Piperine is soluble in ethanol so it was expected that a solution of HCl/Ethanol would serve as a suitable loading solution. Stability of Piperine in HCl was another factor to go with HCl/Ethanol as the loading solution. 25/75, 50/50 and 75/25 HCl/Ethanol solutions with 10mg of drug were used as loading solutions for initial trials. **(Fig. 5S)**. The results showed that the drug release from 25/75 HCl/Ethanol sample was the maximum (around 5mg) after 3 days and still showed a trend towards more release, while the other samples equilibrated with a release of 3-4 mg after 24 h (**Fig. 5S)**. This was an indication that ethanol played a major role in drug loading. As the content of ethanol increased, the solubility of piperine increased. HCl ensured the high swelling of the nuggets, which in turn allowed greater penetration of the soluble drug molecules. So, 25/75 HCl/Ethanol solution was selected for further experiments.

Drug release: Varying the SA coating and Cross-linking degree

Two sets of experiments were carried out, wherein the first one the cross-linking degree (5% $CaCl_2$) was kept constant and the SA coating percentage was varied (0.5 % and 1.5 %) and in the second one, SA coating percentage was kept constant (1%) and the $CaCl_2$ cross-linking degree was varied (2.5 % and 7.5 %). These samples were

studied at pH 7.4 to investigate the factor that could further enhance the control over the drug release.

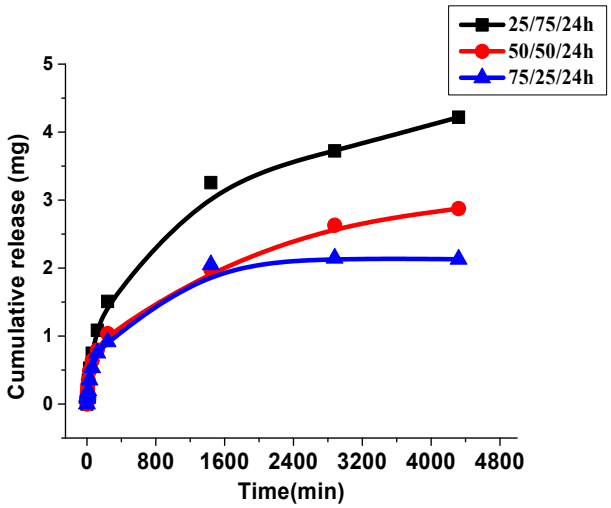

Fig. 5S Cumulative drug release for 25/75, 50/50 and 75/25 HCl/Ethanol piperine loaded samples

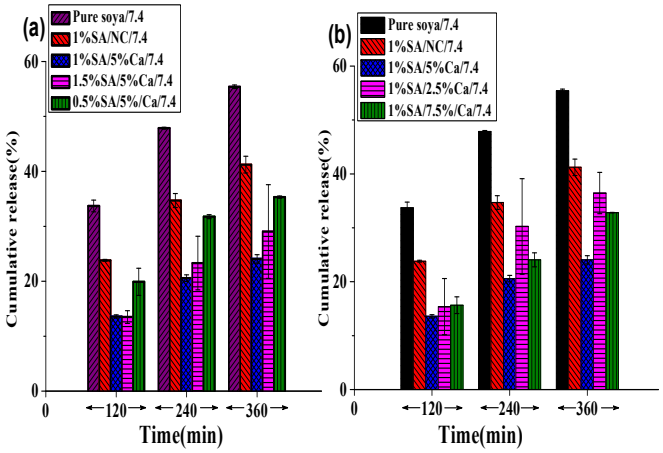

Fig. 6S (a) Cumulative drug release of SA coated samples (1%, 1.5% and 0.5 %) cross-linked with 5% $CaCl_2$ at pH 7.4 (b) Cumulative drug release for 1%SA coated samples cross-linked with 5%, 2.5% and 7.5% $CaCl_2$

Fig. 6S and 7S give a clear picture of the type of interactions occurring based on the concentration of SA and $CaCl_2$. In the first set of experiments, where the cross-linking degree was constant (5% $CaCl_2$) it was observed that 0.5%SA/5% $CaCl_2$ sample gave the highest drug release (among crosslinked samples), as 5% $CaCl_2$ was too high a concentration to cross-link 0.5%SA that resulted in free calcium ions near the surface inviting more aqueous solution that resulted in higher swelling and therefore a high drug release. On the other hand the release of 1.5%SA/5%$CaCl_2$ was higher than 1%SA/5%$CaCl_2$ sample, as extent of crosslinking is less. 1%SA and 5% $CaCl_2$ was the best one as it provided a balance between the degree of crosslinking and free calcium ions **(Fig. 7S)**. Similar results were observed in the second set of experiments where the SA concentration was constant (1%) and $CaCl_2$ concentration was varied (2.5%, 5 % and 7.5 %). 2.5 % $CaCl_2$ turned out to be too less a concentration to cross-link entire SA on the surface of the nugget while 7.5 % $CaCl_2$ resulted in free calcium ions near the surface **(Fig. 7S)**. Thus in both the cases we observe that 1%SA/5% $CaCl_2$ offers a better control over the release of piperine.

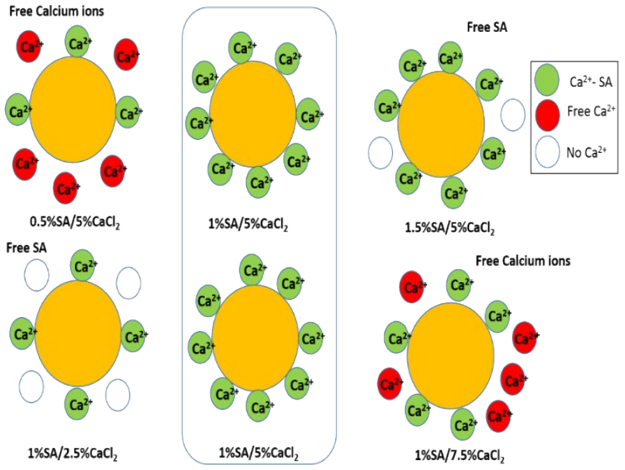

Fig. 7S Interaction of $CaCl_2$ with SA on the surface of soya nuggets

Drug release: Varying the weight of nuggets

The variation in loading efficiency and SD due to the nugget weight triggered another interesting study i.e. the release of piperine from nuggets with varying sizes (weights). As observed that there is an optimum range of nugget weight, which can offer better porosity, swelling and higher loading. So, it was expected that this study would help us in designing a vehicle that can be selected on the basis of the type of release required i.e. fast to slow.

The small nugget pieces were selected on the basis of their weight such that their equivalent weight was equal to an intact nugget. These small pieces were again loaded with drug following the same protocol as mentioned before, coated with 1% SA and cross-linked with 5%$CaCl_2$.

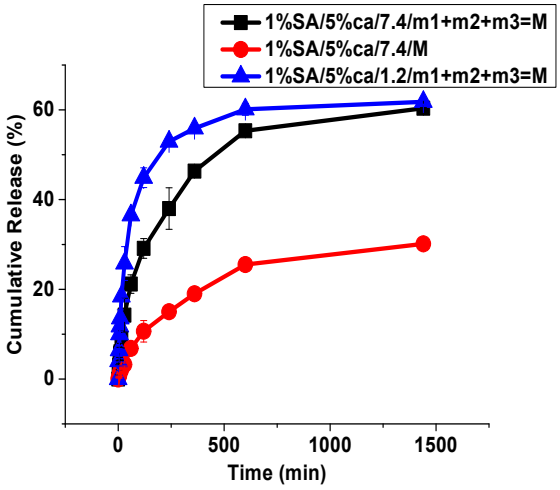

Fig. 8S Drug release study for small sized nuggets

These small nuggets together were immersed in PBS (pH 7.4) and 0.1N HCl (pH 1.2) and studied for drug release. It was observed that the rate of drug release was faster in the sample having small nugget pieces. 20 % of drug release was observed after 1 h in small nuggets while it was only around 7% in the intact one. Similar differences in release rate were recorded after 360 m and 24 h in pH 7.4 **(Fig. 8S)**. The amount of drug release was around 60% in small nuggets while it was only 30 % in intact nugget after 24 h in pH 7.4.

The important observation here was that the release in pH 1.2 for smaller nuggets was more as compared to smaller nuggets in pH 7.4. It was 36% in the first hour 61% after 24 h compared to 20% and 60% in pH 7.4 **(Fig. 8S)**. The faster release of drug in the smaller nuggets at pH 7.4 and 1.2 can be attributed to the decrease in the diffusional length of the sample. As the nugget size decreased, the distance travelled by drug molecule from core to the surface and to the solution also decreased that led to a higher rate of drug release. One more aspect of this study was the amount of drug release. As described before that smaller weight nuggets lead to better swelling and loading, the same fact can further be confirmed by these results. These results confirm the fact that these nuggets can be designed in a way that covers the entire spectrum of drug release. Smaller nuggets can give better loading as well as a faster release while a bigger nugget can offer a much slower and controlled release, however a higher release in pH 1.2 for smaller nuggets also gives an indication that the usage of small nuggets should be limited for drugs intended for release in stomach conditions or having very slow biodegradation.

Soya nuggets: Swelling Kinetics

The swelling of soya nuggets was considered to be second order and the swelling rate at particular time interval was expressed according the **equation S4**:

$$\frac{dW}{dt} = K\left(W_\infty - W\right)^2 \quad \textbf{(Equation S4)}.$$

Integrating eq (1) between t=0 and W=0 we get

$$W = \frac{K(W_\infty)^2 t}{1 + KW_\infty t} \quad \textbf{(Equation S5)}.$$

Rearranging **equation S2** gives **equation S6**:

$$\frac{t}{W} = \frac{1}{K(W_\infty)^2} + \frac{t}{W_\infty} \quad \textbf{(Equation S6)}.$$

According to the given equations the data obtained from the experiments should fit a straight line with a slope of $1/W_\infty$ and intersection point of $1/(K.W_\infty^2)$. Thus from the above equations, we can calculate the water content inside the nuggets at equilibrium (W_∞) and also the initial swelling rate (dW/dt) **(Table 1S)**. The swelling kinetics study for all the samples was considered for 24h.The initial swelling rate of pure soya (non-coated and non-cross-linked) was higher in pH 7.4 but the final swelling degree after 24 h was less in pH 7.4 (SD= 218 (pH=7.4); SD = 315(pH 1.2)). However when the samples were coated and cross-linked there was a marked decrease in the initial swelling rate in pH 1.2.

Drug release kinetics

The equation for Higuchi kinetics is expressed as **equation S7**:

$$\frac{M_t}{M_\infty} = K_H t^{\frac{1}{2}} \quad \textbf{(Equation S7)}$$

Table 2S shows the Higuchi rate constants calculated for the drug release. The results are again governed by two factors i.e. the amount of free calcium present near the surface that invites more initial solution that resulting in higher swelling and the free carboxylate groups of SA that are not cross-linked due to low concentration of $CaCl_2$ **(Fig. 9S)**, that again results in higher swelling as explained in **Fig. 7S.**

Table 1S. Results of swelling kinetics for 24 h swelling study

Sample	pH	W_∞ Experimental	W_∞ calculated	Initial swelling rate X 10^2 (dW/dt)	r^2
Pure soya	7.4	217.61	218.34	0.291	0.99
Pure soya	1.2	312.91	315.45	0.111	0.99
1% SA	1.2	317.70	322.58	0.106	0.99
1%SA 1%$CaCl_2$	1.2	315.18	319.48	0.0841	0.99
1%SA 2%$CaCl_2$	1.2	270.06	273.97	0.0886	0.99
1%SA 3%$CaCl_2$	1.2	310.77	315.45	0.0597	0.99
1%SA 5%$CaCl_2$	1.2	240.34	243.30	0.0642	0.989

Table 2S. Drug release kinetics: Higuchi rate constants

Sample	pH	K_H	r^2
Pure Soya	7.4	2.92	0.99
Pure soya	1.2	2.15	0.99
1%SA Coated	7.4	2.09	0.98
1%SA Coated	1.2	1.37	0.99
1% SA 5% $CaCl_2$	7.4	1.22	0.98
1% SA 5% $CaCl_2$	1.2	1.19	0.99
1% SA 2.5% $CaCl_2$	7.4	1.75	0.97
1% SA 7.5% $CaCl_2$	7.4	1.49	0.95
1.5% SA 5% $CaCl_2$	7.4	1.36	0.96
0.5% SA 5% $CaCl_2$	7.4	1.84	0.98

Soya Nuggets: Diffusion Kinetics

The swelling of soya nuggets results from the diffusion of water molecules into the nuggets. The inflow of water firstly creates open spaces i.e. swelling and then the water molecules starts to diffuse into the pores of soya nuggets. To determine the nature of diffusing water molecules, the following **equation S8** was used:

$$\frac{M_t}{M_\infty} = K\, t^n \qquad \textbf{(Equation S8)}$$

In the above equation M_t is the amount of solvent diffused at time t, M_∞ is the amount of solvent diffused at infinite time, K is a constant depicting the structure of the network and n is the characteristic constant that actually identifies the mode of solvent diffusion.

Depending on the relative rates of diffusion and polymer relaxation, three classes of diffusion mechanisms are identified, assuming soya nuggets to be spherical in shape.

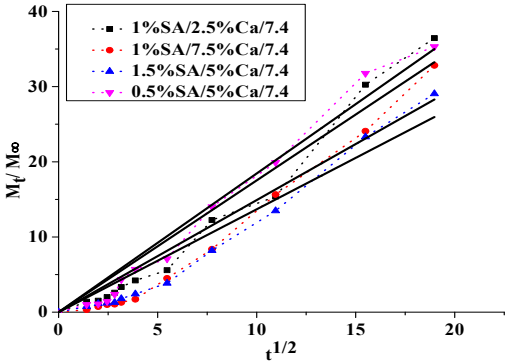

Fig. 9S Release kinetics: Cumulative release vs $t^{1/2}$.

Fickian diffusion in which the rate of diffusion is much less than that of relaxation (n = 0.43); Zero order, in which diffusion is very rapid compared with the relaxation processes (n = 1) and Non-Fickian or anomalous diffusion, which occurs when the diffusion and relaxation rates are comparable (0.43 < n < 1).When the water penetration rate is much below the polymer chain relaxation rate, it is possible to record the n values below 0.43. This situation can be referred to as less Fickian behavior. In the case of soya nuggets, less Fickian case was encountered for all the cases studied. n values ranged from 0.17-0.326. **(Table 3S).** As the swelling increases, k decreases. The same trend was followed by pure soya samples and coated and cross-linked samples up-to 2%. Slight increase was observed in the k value for 3% and 5%, which can be due to the free calcium present on the surface of the nuggets. Similar deviation was also found in the swelling kinetics where a slight increase was observed in the kinetic constant values for 3% and 5% cross-linked sample. The experimental and predicted values have been compared in **Fig. 10S (a)** and **Fig. 10S (b)**.

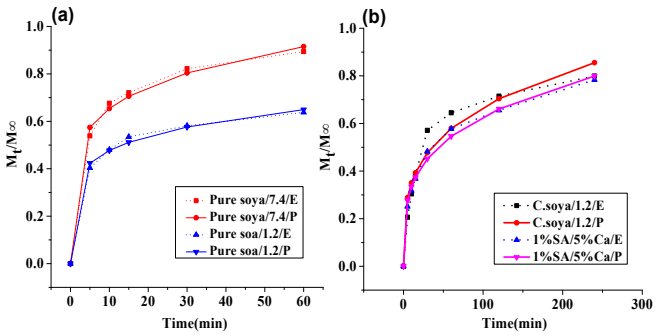

Fig. 10S (a) Diffusion kinetics of pure soya sample, experimental and predicted (E & P) in pH 7.4, 1.2 (b) Diffusion kinetics of coated soya sample (C. soya), and 5% $CaCl_2$ cross-linked, experimental and predicted (E & P) pH 1.2

Table 3S. Swelling Degree: Diffusion Kinetics

Sample/pH	Time (min)	K	n	Type of diffusion
Pure soya/7.4	60	0.42	0.18	Less Fickian
Pure soya/1.2	60	0.32	0.17	Less Fickian
1%SA/1%$CaCl_2$/1.2	240	0.137	0.326	Less Fickian
1%SA/2%$CaCl_2$/1.2	240	0.157	0.313	Less Fickian
1%SA/3%$CaCl_2$/1.2	240	0.148	0.28	Less Fickian
1%SA/5%$CaCl_2$/1.2	240	0.17	0.24	Less Fickian

Table 4S. Isoelectric pH of Essential amino acids

Essential Amino Acid	pI (Isoelectric pH)
Histidine	7.64
Leucine	6.04
Isoleucine	6.04
Methionine	5.74
Phenylalanine	5.91
Tryptophan	5.88
Valine	6.02
Threonine	5.60
Lysine	9.47

Storage of Drug loaded soya nuggets

Soya nuggets were aimed at achieving a controlled oral drug release, so it was important that they remain soft and spongy to aid easy swallowing. To maintain this nature of drug loaded swelled nuggets, they were wrapped in aluminium foil and stored in refrigerator at 4°C for over 30 days. It was observed that even after 30 days the nuggets was soft and spongy and could retain the aqueous medium inside them **(Fig. 11S)**.

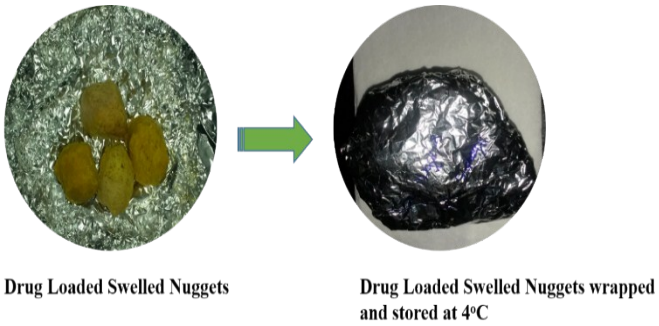

Fig. 11S Storage of drug loaded soya nuggets