Journal of Science and Technology
ISSN: 2456-5660 Volume 6, Issue 04 (July -Aug2021)
www.jst.org.in *DOI:https://doi.org/10.46243/jst.2021.v6.i04.pp292- 301*

Design and In-vivo Assessment of Quercetin-Based Nanosponges Buccal Quercetin Tablets

Dr.Ratnasree [1],Dr.Rafia [2],Shivaprasad [3],Noureen[4,]
Assistant professor [1,2,3,4],
Department of Pharmacy,
Samskruti College of Pharmacy,
Kondapur (V), Ghatkesar (M) Medchal Dist, Telangana, India

To Cite this Article

Dr.Ratnasree [1],Dr.Rafia [2],Shivaprasad [3],Noureen[4,]" Design and In-vivo Assessment of Quercetin-Based Nanosponges Buccal Quercetin Tablets " *Journal of Science and Technology,*

Vol. 06, Issue 04,- July-Aug 2021, pp292-301

Article Info

Received: 04-06-2021 Revised: 05-07-2021 Accepted: 15-07-2021 Published: 27-07-2021

ABSTRACT

The goal was to use cyclodextrin-based nanosponges to create a controlled release formulation that would boost quercetin's bioavailability. Using the freeze-drying method, a 3-factor, 3-level Box-Behnken design containing quercetin was loaded into nanosponges based on the results of preliminary testing. After being manufactured, characterized, and formed into tablets, the prepared nanosponges were inspected. The drug release percentages at six hours range from 53.04 to 82.64% for the quercetin-loaded nanosponges, whereas the particle sizes range from 36.45 to 135.27 nm and the encapsulation efficiencies from 42.37 to 88.44%. The Quercetin-nanosponge interaction was confirmed by FTIR, DSC, and XRD analyses. After the nanosponges were converted into tablets, the medication released from them at a rate of 99.75% in vitro, and stability tests revealed no appreciable alterations six months later. Rats were used in in vivo investigations to compare the Cmax of quercetin optimized nanosponges tablets (6.27 ± 0.06 ng/mL) to the Cmax of the pure medication (3.07 ± 0.086 ng/mL), which was substantially lower (p<0.05). The Tmax values for the pure drug solution and the nanosponges tablet formulation were 0.5 ± 0.08 h and 4.0 ± 0.07 h, respectively.

Introduction

Of all the flavonoids discovered to date, quercetin (3,3',4',5,7-pentahydroxy-f lavone) is the largest member of the flavonol subclass. Among other biological and pharmacological effects, it has been shown to have anti-cancer, anti-oxidation, anti-inflammatory, blood cholesterol-lowering, coronary artery dilation, anti-platelet aggregation, anti-anemia, and antianaphylaxis qualities.[1] Nevertheless, quercetin is a challenging molecule to deliver therapeutically because of its poor solubility, low hydrophilicity (log p-value of 1.81), gastrointestinal instability, high first-pass metabolism, and little absorption in the gastrointestinal tract. Quercetin is classified as a class II BCS substance.[2] It dissolves at 7.7 in water, 5.5 lg/mL in gastric simulated fluid, and 28.9 lg/mL in intestinal simulated fluid (SIF). The medication's therapeutic usage in conventional dosage forms is limited due to its oral bioavailability, which has been shown to be less than 17% in rats and even less than 2% in humans.[3] As a result, a more potent form of quercetin that has improved absorption and action is required. Regarding the many drug delivery methods that have been documented in the literature, quercetin nanoparticulate formulation seems to be a good choice for simultaneously enhancing stability and solubility. Recently, cyclodextrin polymers hypercross-linked and nanostructured to produce three-dimensional networks have been converted into nanosponges. Cyclodextrin is reacted with an appropriate crosslinking agent, such as diphenyl carbonate or carbonyl diimidazole, to create the nanostructured materials. Natural

cyclodextrins were not as good in complexing with a wide range of compounds as cyclodextrin-based nanosponges. They have been used to protect the labile groups, regulate release, and improve the solubility of poorly soluble actives.[4] Over the last 10 years, a great deal of research has been done on buccal mucoadhesive dosage forms because of their distinct physiological properties. One might use the buccal route for systemic as well as local administration. Convenient administration of these formulations at the sites of illness might minimize adverse effects, enhance patient adherence, and demonstrate long-term retention at the targeted site of action. In this work, we used cyclodextrin nanosponges as unique nanocarriers to create nanosponges integrated with buccal tablets containing quercetin.

Materials And Methods

Materials

Of all the flavonoids discovered to date, quercetin (3,3',4',5,7-pentahydroxy-f lavone) is the largest member of the flavonol subclass. Among other biological and pharmacological effects, it has been shown to have anti-cancer, anti-oxidation, anti-inflammatory, blood cholesterol-lowering, coronary artery dilation, anti-platelet aggregation, anti-anemia, and antianaphylaxis qualities.[1] Nevertheless, quercetin is a challenging molecule to deliver therapeutically because of its poor solubility, low hydrophilicity (log p-value of 1.81), gastrointestinal instability, high first-pass metabolism, and little absorption in the gastrointestinal tract. Quercetin is classified as a class II BCS substance.[2] It dissolves at 7.7 in water, 5.5 lg/mL in gastric simulated fluid, and 28.9 lg/mL in intestinal simulated fluid (SIF). The medication's therapeutic usage in conventional dosage forms is limited due to its oral bioavailability, which has been shown to be less than 17% in rats and even less than 2% in humans.[3] As a result, a more potent form of quercetin that has improved absorption and action is required. Regarding the many drug delivery methods that have been documented in the literature, quercetin nanoparticulate formulation seems to be a good choice for simultaneously enhancing stability and solubility. Recently, cyclodextrin polymers hypercross-linked and nanostructured to produce three-dimensional networks have been converted into nanosponges. Cyclodextrin is reacted with an appropriate crosslinking agent, such as diphenyl carbonate or carbonyl diimidazole, to create the nanostructured materials. Natural cyclodextrins were not as good in complexing with a wide range of compounds as cyclodextrin-based nanosponges. They have been used to protect the labile groups, regulate release, and improve the solubility of poorly soluble actives.[4] Over the last 10 years, a great deal of research has been done on buccal mucoadhesive dosage forms because of their distinct physiological properties. One might use the buccal route for systemic as well as local administration. Convenient administration of these formulations at the sites of illness might minimize adverse effects, enhance patient adherence, and demonstrate long-term retention at the targeted site of action. In this work, we used cyclodextrin nanosponges as unique nanocarriers to create nanosponges integrated with buccal tablets containing quercetin.

S. No.	*Type of NS*	*Molar ratio (β-CD: DPC)*	*Concentration of β-cyclodextrin (g)*	*Concentration of diphenyl carbonate (g)*
1	NS1	1:2	4.548	1.712
2	NS2	1:4	4.548	3.424
3	NS3	1:6	4.548	5.136
4	NS4	1:8	4.548	6.848
5	NS5	1:10	4.548	8.560

Optimization

The optimization approach was validated by preparing the nanoformulation in triplicate under ideal circumstances.[9] Features of Ready-Made Quercetin Nanosponges The method used for β-cyclodextrin nanosponges was followed in order to estimate the particle size, polydispersity index, and zeta potential. The formulations underwent FTIR, DSC, and PXRD analyses in accordance with the methodology used in the reference.[10]

Description of Ready-to-Use Quercetin Nanosponges Equations 1 and 2 were used to get the "percent drug payload" and "percent drug encapsulation efficiency."

$$\% \text{ Drug pay load} = \frac{\text{Weight of drug encapsulated in NS formulation}}{\text{Weight of the NS formulation taken for analysis}} \times 100 \quad (1)$$

$$\% \text{ Drug encapsulation efficiency} = \frac{\text{Weight of drug encapsulated in NSformulation}}{\text{Initial weight of the drug fed for loading}} \times 100 \quad (2)$$

Preparation of Quercetin Loaded Nanosponges Buccal Tablets

Using a mortar and pestle, precisely weighed quantities of quercetin-loaded nanosponges (equivalent to 100 mg quercetin) and the calculated Avicel PH-102—which was added to achieve a 300 mg tablet—were mixed for 10 minutes. Next, 6 mg of magnesium stearate was added, and the mixture was blended for an additional 2 minutes. The final mixes were crushed using an 8 mm round, flat-faced single punch single punch tablet machine.[11]
Assessment of Tablet Formulation
Weight homogeneity, drug content, friability, hardness, and in vitro disintegration tests.[11,12]
Quercetin In-vitro Release Investigation

Using the type II USP dissolving equipment, in-vitro r elease o f d rug f rom quercetin p ure d rug, quercetin NS powder, and quercetin NS loaded tablets was carried out.[12] For the first two hours, the dissolving medium consisted of 900 mL of 0.1 N HCl, which was soon changed to phosphate buffer pH 6.8 at a speed of 50 rpm at a temperature of 37 ± 0.5°C. At 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours, the samples were taken out. Immediately after, the same volume of brand-new dissolving medium was added and kept at the same temperature. The materials were examined at 369 nm using a UC spectrophotometer after being appropriately diluted. Three separate dissolving tests were carried out.

Studies of Short-Term Stability
In compliance with ICH requirements, stability investigations of the improved formulation were conducted. After the quercetin buccal tablets were filled and sealed in light-colored, protective bottles with rubber stoppers and aluminum coatings, their stability was assessed. The samples were taken out at predetermined intervals and their appearance, hardness, disintegration time, dissolution, and drug content were all examined. These were stored at three different temperatures and relative humidity levels (i.e., 25 ± 2°C, 60% ± 5; 30 ± 2°C, 65% ± 5; and 40 ± 2°C, 65% ± 5).

Results and Discussion

Various molar ratios of reactants were used to create five distinct kinds of nanosponges.[15] Following measurement, the following values are shown in Table 4: zeta potential, particle size, polydispersity index, and percent practical yield. The polymer to cross-linker ratio range of 0.2–0.8, the stirring speed of 2000–5000 rpm, and the stirring duration of 350–550 minutes were determined from the experiments. To maximize the contributing factors, a Box-Behnken design was used, taking into account the preliminary findings.[15]

Average Particle Dimensions

The nanoformulation's particle sizes vary from 36.45 to 135.27 nm.[16] Figs. 1a and b illustrate the interaction influence of AB on particle size at a constant level of C.

Efficiency of Encapsulation

It was discovered that the nanosponges' encapsulation effectiveness ranged from 42.37 to 88.44% (Table 2). Figs. 2a and b illustrate how BC interacts with encapsulation efficiency at a fixed amount of A.

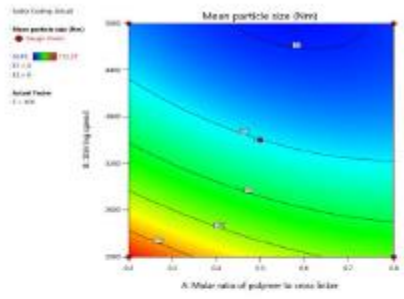
Drug Release Percent at Six Hours

An essential metric to evaluate nanosponges' efficacy in regulating medication release for a predetermined duration is the percentage of drug release at 6 hours. Table 2 shows that the range of drug release percentages from the nanoformulation is 53.04 to 82.64%.

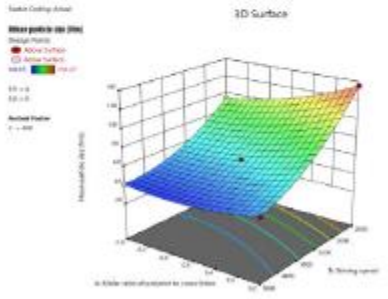
According to the polynomial model, only the variable

Table 2: BBD lists the independent and dependent variables along with the corresponding objectives and levels.

Independent variables			*Levels*		
Variable		*Units*	*Low*	*Intermediate*	*High*
A	Molar ratio of polymer to cross linker		0.2	0.5	0.8
B	Stirring speed	rpm	2000	3500	5000
C	Stirring time	Min	350	450	550
Dependent variables			*Goal*		
Y1	Mean particle size	Nm	Minimize		
Y2	Encapsulation efficiecny	%	Maximize		
Y3	Percent drug release at 6h	%	Minimize		



(a)



(b)

Fig. 1: (A) Two-dimensional contour plot illustrating the interaction between A and B on the mean particle size at a fixed C level. (b). Plot of the 3D response surface illustrating the interaction between A and B on the mean particle size at a fixed level of C. An important factor influencing the percentage of medication released from nanosponges was a (Molar ratio). A perturbation plot was used to illustrate how variable A affected Y3 (Fig. 3).
Enhancement Utilizing Derringer's desire function (D), the chosen factors that affect the response parameters were optimized. (Tabel 5).[17] Sizes and Shapes of the Nanosponges Loaded with Quercetin The average particle size of the quercetin-loaded nanosponges, as determined by the laser light scattering technique, is around 40 to 50 nm, with a low polydispersity index, according to the particle size analysis. The distribution of particle sizes

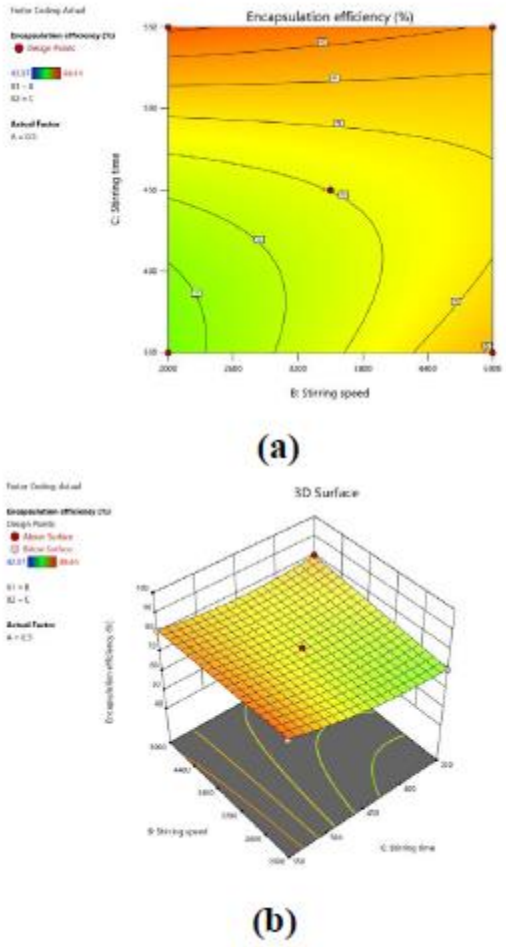


Fig. 2: (a) 3D- Contour plot showing the interactive effect of B and C on encapsulation efficiency at constant level of A. (b). 3D- response surface plot showing the interactive effect of B and C on encapsulation efficiency at constant level of A.

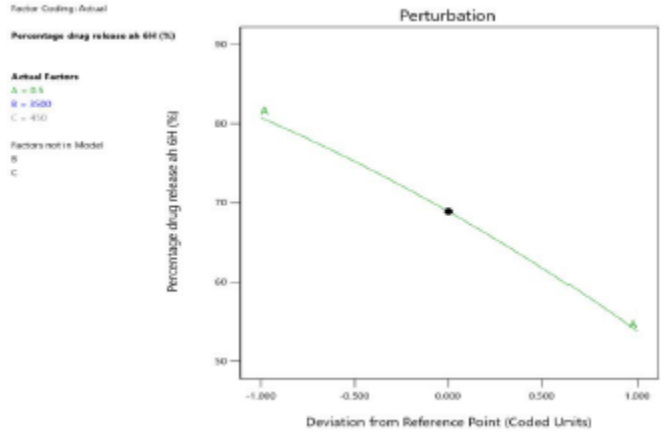


Fig. 3: Table 6 illustrates the unimodal and limited effect of A on the % drug release at 6 hours in a two-dimensional perturbation plot. A low polydispersity index indicates the homogeneity of the colloidal particles. If the zeta potential is high enough, the complexes should be stable and have very little inclination to clump together.
It was discovered that all of the created formulations were fine, free-flowing powders. Table 6 shows the percentage medication loading and encapsulation effectiveness of the produced quercetin nanosponges.
Table 3: Trial experiment observations according to BBD

Expt	*Molar ratio of polymer to cross linker*	*Stirring speed (rpm)*	*Stirring time (min)*	*Mean particle size (nm)*	*Encapsulation efficiecny (%)*	*Percent drug release at 6h (%)*
1	0.5	3500	450	68.25	90.82	60.28
2	0.2	5000	450	60.02	57.12	79.82
3	0.5	3500	450	69.49	90.18	60.92
4	0.5	5000	350	68.37	86.32	62.22
5	0.2	3500	550	63.14	57.88	79.12
6	0.5	2000	350	153.2	84.23	68.23
7	0.5	5000	550	52.27	93.21	60.54
8	0.5	2000	550	147.38	89.74	65.69
9	0.2	2000	450	153.42	53.44	83.11
10	0.8	2000	450	155.28	76.54	55.18
11	0.8	3500	550	81.77	79.82	54.11
12	0.5	3500	450	73.65	91.34	59.86
13	0.8	3500	350	79.21	76.56	54.45
14	0.8	5000	450	62.84	75.12	52.34
15	0.5	3500	450	69.33	90.88	59.76
16	0.5	3500	450	70.9	91.86	60.34
17	0.2	3500	350	89.68	54.13	81.05

Table 4: The percent practical yield, particle size, polydispersity index and zeta potential of different nanosponges

S. NO.	*Type of NS*	*Molar ratio (β-CD: DPC)*	*Practical yield (%)*	*Mean particle size (nm)*	*Polydispersity index*	*Zeta potential*
1	NS1	1:2	77.64 ± 2.76	111.96 ± 3.52	0.251 ± 0.005	-22.64 ± 2.12
2	NS2	1:4	82.27 ± 1.98	107.21 ± 4.88	0.308 ± 0.005	-25.16 ± 1.13
3	NS3	1:6	85.82 ± 3.12	115.67 ± 3.42	0.262 ± 0.005	-26.38 ± 3.24
4	NS4	1:8	90.35 ± 2.44	120.28 ± 4.26	0.418 ± 0.005	-23.02 ± 1.74
5	NS5	1:10	92.48 ± 1.89	99.33 ± 2.48	0.270 ± 0.005	-22.48 ± 1.46

(All determinations were performed in triplicate and values were expressed as mean ± S.D.,n = 3 ($p < 0.05$)

Table 5: Optimum conditions attained by applying restrictions on response parameters

Independent variables	*Optimized values*	*Predicted values*			*Actual values*			
		Mean particle size (Y_1) Nm	*Encapsulation efficiency (Y_2) %*	*Percent drug release at 6h (Y_3)*	*Batch*	*Mean particle size (Y_1) nm*	*Encapsulation efficiency (Y_2) %*	*Percent drug release at 6h (Y_3)*
Molar ratio of polymer to cross linker	0.80				F1	40.62 ± 4.62	87.06 ± 1.67	55.50 ± 1.28
		36.831	85.991	53.813	F2	46.39 ± 4.19	86.27 ± 2.49	56.04 ± 2.17
Stirring speed	5000							
					F3	48.21 ± 2.50	87.60 ± 1.28	56.75 ± 1.05
Stirring time	525 min							

n = 3 ($p < 0.05$)

Characterization of Cyclodextrin Nanosponges

No significant interactions were verified by FTIR and DSC experiments, and the XRPD research confirmed quercetin's creation of an inclusion complex with nanoponges and complete loss of crystallinity.[18]

Making Baccal Tablets with Quercetin Loaded Nanosponges.

The weight distribution was 300.46 ± 2.27 to 301.97 ± 3.56 mg for the mean. The range of the mean thickness is 4.95 ± 0.46 to 5.19 ± 0.31 mm. The range of the mean hardness is 5.31 ± 0.38 to 5.45 ± 0.49 kg/cm2. Table 7 displays the average percentage drug content ranging from 98.84% ± 1.76 to 99.61 ± 1.19, and the mean friability values ranging from 0.51 ± 0.24 to 0.79% ± 0.18.[19, 20] Table 8 revealed that T2 had the highest swelling index. All of the formulations provide an adequate pH in the salivary pH range of 5 to 7, as shown by the surface pH values, which ranged from 6.5 to 6.6. (Table Nine).[21] The mucoadhesion of buccal tablets was 19.26, 20.46, and 22.95 g, in that order (Table 9). The duration of residence for buccal tablets ranged from 6.4 to 6.7 hours, suggesting that the tablets need this amount of time to be eliminated from the buccal mucosa.

Study of In-vitro Release

The dissolving patterns of quercetin in pure drug solutions, as well as from various quercetin nanosponges powder and buccal tablet formulations (Fig. 4). Quercetin from the produced nanosponges buccal tablets released in a biphasic manner. 17.64% of the medication was released in an initial burst within an hour, and the remaining drug was released continuously for a whole day after that. After 24 hours, 99.75% of the quercetin contained in the buccal tablets of nanosponges was released.[22, 23]

Studies of Short-Term Stability

As shown in Table 10, the stability study's findings showed that the drug content, dissolving rate, hardness, disintegration time, and visual appearance had not changed significantly.[24, 25]

Table 6: Zeta potential, polydispersity index, and particle size of drug-loaded and plain nanosponge formulations

Sample	*Mean particle size ± SD (nm)*	*Polydispersity Index*	*Zeta potential (mV)*	*Drug pay load*	*Encapsulation efficiency*
Plain NS	108.24 ± 3.67	0.30 ± 0.005	-21.37 ± 1.12	-	-
F1	41.36 ± 4.32	0.44 ± 0.005	-20.7 ± 1.62	48.15	87.88 ± 1.08
F2	46.9 ± 3.72	0.12 ± 0.005	-23.04 ± 1.74	49.37	86.73 ± 1.65
F3	48.72 ± 4.51	0.32 ± 0.005	-24.68 ± 1.19	48.02	87.64 ± 3.27

n = 3 ($p < 0.05$)

Table 7: Evaluation parameters of quercetin tablets

Formulation	*Weight (mg)*	*Thickness (mm)*	*Hardness (kg/cm²)*	*Friability (%)*	*Drug content (%)*
T1	300.46 ± 2.27	4.95 ± 0.46	5.31 ± 0.38	0.51 ± 0.24	98.84 ± 1.76
T2	301.97 ± 3.56	5.06 ± 0.77	5.45 ± 0.49	0.67 ± 0.52	99.61 ± 1.19
T3	300.62 ± 4.27	5.19 ± 0.31	5.38 ± 1.32	0.79 ± 0.18	99.22 ± 2.61

n = 3 ($p < 0.05$)

Table 8: Swelling index of quercetin nanosponges loaded buccal tablet

Formulation no	*Time (hours)*					
	1	*2*	*3*	*4*	*5*	*6*
T1	50.62	52.91	59.88	61.81	65.75	68.21
T2	65.21	68.65	71.22	74.38	78.60	84.64
T3	52.34	55.29	58.69	62.26	69.27	71.49

n = 3 ($p < 0.05$)

Table 9: Surface pH, Mucoadhesive strength and *ex-vivo* residence time of quercetin nanosponges loaded buccal tablet

Formulation code	*Surface pH*	*Mucoadhesive strength (g)*	*Ex-vivo residence (hours)*
T1	6.6 ± 0.02	19.26 ± 0.62	6.4 ± 0.72
T2	6.5 ± 0.04	20.46 ± 0.76	6.5 ± 0.33
T3	6.6 ± 0.06	22.95 ± 0.27	6.7 ± 0.85

n = 3 ($p < 0.05$)

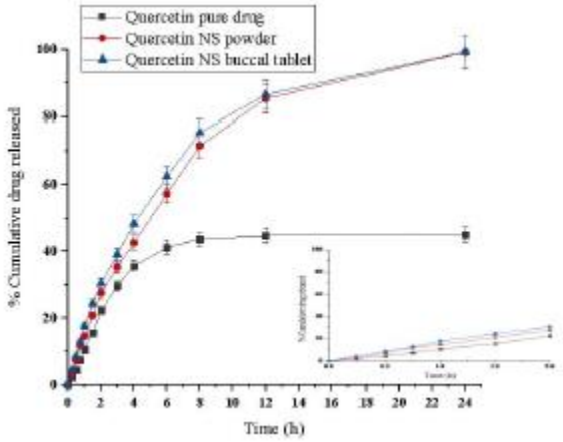


Fig. 4: *In-vitro* release of Quercetinpure drug, quercetin NS powder and quercetin NS buccal tablet [n = 3 ($p < 0.05$)]
Table 11: Pharmacokinetic Parameters of quercetin optimized nanosponges buccal tablets formulation and pure drug

Pharmacokinetic parameters	*Quercetin pure drug*	*Quercetin optimized nanosponges buccal tablets*
C_{max} (µg/mL)	3.07 ± 0.086	6.27 ± 0.06
AUC_{0-t} (µg.h/mL)	6.3275 ± 1.27	37.61 ± 2.28
AUC_{0-inf} (µg.h/mL)	7.84 ± 1.08	38.54 ± 0.65
T_{max}(h)	0.5 ± 0.08	4.0 ± 0.07
$t_{1/2}$ (h)	11.129 ± 1.68	15.41 ± 1.46

This work used the freeze-drying process to create quercetin-loaded nanosponges. It was confirmed by FTIR, DSC, and XRD analyses that the use of nanosponges led to the formation of a quercetin inclusion complex. Due to intermolecular hydrogen bonding, the induction of a high-energy amorphous state, and the decreased drug particle size, the quercetin nanosponges' dissolution was much greater than that of the pure drug. The relative dissolving rate of quercetin nanosponges buccal tablets was 99% when compared to pure quercetin. The Cmax of the nanosponges tablet, 6.27 ± 0.06 ng/mL, was significantly higher ($p<0.05$) than the Cmax of the pure medication, which was 3.07 ± 0.086 ng/mL. The Tmax values for the pure drug and the nanosponges tablet formulation were 0.5 ± 0.08 h and 4.0 ± 0.07 h, respectively. The AUC0-∞ of the nanosponges tablet formulation was greater than that of the pure drug suspension formulation (7.84 ± 1.08 ng.h/mL) at 38.54 ± 0.65 ng.h/mL). Higher blood drug concentrations compared to the pure medication demonstrated superior quercetin systemic absorption from the nanosponges buccal tablet formulation.

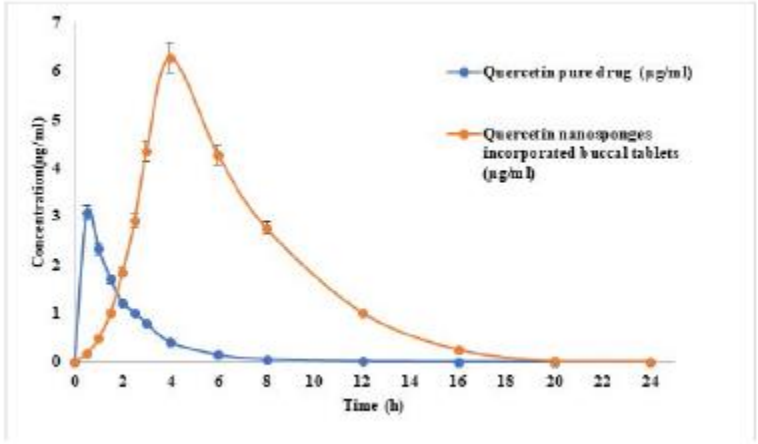


Fig. 5: Plasma concentration profiles of quercetin optimised nanosponges buccal tablets and pure drug

References

1. Hollman PC, Katan MB. Dietary flavonoids: intake, health effects and bioavailability. Food Chem Toxicol. 1999; 37:937–942.
2. Chen-yu G, Chun-fen Y, Qi-lu L et al. Development of a quercetinloaded nanostructured lipid carrier formulation for topical delivery. Int J Pharm. 2012; 430:292–298.
3. Kumari A, Yadav SK, Pakade YB, Singh B, Yadav SC. Development of biodegradable nanoparticles for delivery of quercetin. Coll Surf B Biointer. 2010; 80:184–192.
4. Kaur, Simranjot & Kumar, Sandeep. The Nanosponges: An Innovative Drug Delivery System. Asian J Pharm Chem Res. 2019; 12:60-67.
5. Swaminathan S, Vavia PR, Trotta F, Torne S. Formulation of betacyclodextrin based nanosponges of itraconazole. J Inc Phenom Macrocycl Chem. 2007; 57:89-94.

6. Swaminathan S, Pastero L, Serpe L, Trotta F, Vavia P, Aquilano D, Trotta M, Zara G, Cavalli R. Cyclodextrin-based nanosponges encapsulating camptothecin: physicochemical characterization, stability and cytotoxicity. Eur J Pharm Biopharm.2010; Feb 1; 74(2):193-201.
7. Anandam S, Selvamuthukumar S. Fabrication of cyclodextrin n a no s p on g e s f or quer c e t i n del i ver y : phy s ic o c hem ic a l characterization, photostability, and antioxidant effects. J Mater Sci. 2014; Dec; 49(23):8140-53.