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COMPARATIVE IN VITRO RELEASE STUDIES OF DUAL ENCAPSULATION TECHNOLOGY (DELT) BERBERINE WITH CONVENTIONAL LIPOSOMAL BERBERINE AND UNFORMULATED BERBERINE

Sreya E. S.[1*], Jenish M. John[2], Nandu Bhaskar A. V.[1], Manus Varghese N. J.[2]

[1]R&D Center, Encapscifi Life Sciences Pvt Limited, Bangalore- 560105.
[2]R&D and QC Laboratory, AmSpec Middle East-Hamriyah, Sharjah- 53260.

***Corresponding Author: Sreya E. S.**

R&D Center, Encapscifi Life Sciences Pvt Limited, Bangalore- 560105.

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ABSTRACT

Berberine Hydrochloride (BRN) is poor water soluble alkaloid, used in various traditional medicinal practices. Liposomal BRN formulations have higher oral absorption due to higher solubility but they have low physical and chemical stability. So Dual Encapsulation Technology (DELT) based liposomal BRN is an advanced liposomal technology in which the plant proteins are used to make a surface coating of normal liposomes to get additional stability. In this study we compared the in vitro release of DELT-BRN with conventional liposomal BRN and unformulated BRN. The results showed that DELT-BRN has better release in comparison with conventional liposomal BRN and unformulated BRN.

KEYWORDS: Liposomal Berberine; DELT Berberine; in vitro release; Dialysis membrane.

INTRODUCTION

Liposomes are delivery vehicles that efficiently transfer active molecules into the body by promoting absorption through the intestinal barrier.[1] It's a technique for encapsulating active ingredients inside liposomes, which are tiny, spherical structures made of a lipid bilayer that mimics cell membranes.[2] Biomolecules in liposomal form may improve absorption and delivery by imitating the body's own transport system.[3] As a suitable encapsulation medium with polar- nonpolar amphiphilic phospholipid bilayers, phospholipids can efficiently move hydrophilic (water-soluble molecules like vitamin C, B vitamins and glutathione) and lipophilic (water-insoluble molecules like Curcumin, BRN and Resveratrol) substances through the gastrointestinal (GI) tract's membrane, increasing its permeability.[4]

Furthermore, by temporarily disrupting cellular lipophilic bilayers, biodegradable lipid molecules might enhance transcellular transport and perhaps advance paracellular drug trafficking.[5] The stability, bioavailability, and controlled release of active biomolecules within the gastrointestinal tract are all improved by liposomal encapsulation.[4] With greater bioavailability, distribution, longer circulation duration, improved solubility, decreased toxicity, and improved stability, liposomal delivery systems present a promising option for the delivery of biomolecules.[3]

However, traditional liposomal formulations are prone to rapid clearance from the bloodstream and have other disadvantages that compromise the therapeutic efficacy of active biomolecules.[3] Similarly, phospholipids may aggregate, changing the size of the vesicle, causing drug seepage due to the delicate lecithin membrane, or damaging the biomolecules that are enclosed.[2] However, when we store the formulation for a long period, the physical and chemical instability of liposomes causes issues as previously mentioned.[2] Most liposomal formulations in the market are paste or liquid-based, and they may not be particularly stable due to hydrolysis, lipid peroxidation, flocculation, and aggregation since liposomes break readily in liquid formulations due to the delicate lipid surface.[6] To overcome these difficulties many companies introduced powdered liposomes, but there too having some problems like the deformation of the liposomal structure and degradation of the delicate surface of the lecithin due to the drying.[7]

Therefore, to provide a surface wall to the lecithin with processed plant proteins to make a durable surface membrane will be a novel way to improve liposome stability by modifying the lecithin's surface, which will also provide the liposomes more resilience against degradation.[8] Processed proteins provide an additional layer of protection for lecithin, preventing biomolecules from leaking out of the liposomal core.[9]

In that regard, plant protein-based surface modified liposomes, which converts the liquid liposomes to a novel stabilized form of powdered liposomes.[2] Since liposomes break easily in liquid formulations due to hydrolysis and peroxidation, the typical liposomal solutions on the market are in liquid or paste form, which may not have much stability.[6] Although the powder form can conceal these issues, it is also difficult to maintain the spherical structure since liposomes are prone to breaking during the evaporation of water, and the lecithin surface is fragile and rapidly breaks.[10] This can be avoided by using processed plant protein to create an additional surface wall that maintains the liposomal bilayer structure in powdered form.[9]

Through the surface modification, the processed plant protein is properly integrated with the lecithin by in-situ complexation due to the cationic-anionic interactions with the lecithin, protecting the spherical structure and increasing the liposomes' stability in stomach acids.[11] Also, we ensure the surface coating of the lecithin by the advanced layer-by-layer coating technology at controlled conditions like pH, temperature, and monitored by sophisticated analytical techniques.[1] Additionally, this powdered formulation might shield the bioactive molecules against deterioration, hydrolysis, aggregation, and leaching out from the core structure—all of which are frequent occurrences in liquid liposomes.[7] Thus, processed protein-lecithin delivery systems efficiently carry biomolecules into the body by either enhancing intestinal absorption or avoiding stomach acid breakdown.[12] Additionally, the final formulations are spherical in shape, less angular, and much smoother when processed plant proteins are used.[7] Therefore, smaller angles and smoother surfaces reduce the angle of contact with water and intestinal wall, increasing dispersion in water and permeability through intestinal wall, respectively.[12]

Hence, one novel approach to improving the stability of conventional liposomes is by using plant- based proteins to reinforce lecithin by dual encapsulation of biomolecules like BRN, that renders extra stability to the liposomal surface by the surface modification, hence protecting it from the degradation. In that regard, plant protein-based surface modified liposomes called Dual Encapsulated Liposmal technology (DELT), which converts the liquid liposomes to a novel stabilized form of powdered liposomes. Here we analyse the in vitro release of the DELT BRN in comparison with conventional liposomal BRN and unformulated BRN.

MATERIALS AND METHODS

DELT-BRN conventional liposomal BRN and BRN were obtained from Encapscifi Life Sciences, Bangalore. Dialysis Tubing cellulose membrane (Flat width -28.46 mm; diameter - 17.5 mm,) was received from Sigma Aldrich with molecular weight cut-off (MWCO) 12 KDa was activated using manufacturer's protocol. All other solvents for analysis and studies were purchased from Merck, India.

The comparative in vitro release study of DELT-BRN, conventional liposomal BRN and the unformulated BRN was conducted using the dialysis set up. A. Prior to the diffusion experiment, the dialysis membrane was soaked in phosphate buffer saline (PBS, pH 7.4) for 12 h. A weighed quantity of DELT-BRN (50 mg) was suspended in 10 mL of PBS and transferred into the dialysis bag (donor compartment). The dialysis bag was placed in a beaker containing 100 mL of PBS (receptor compartment) and was incubated at 120 rpm for 8 h at 37 °C in a shaker incubator.

Aliquots of 1 mL PBS in the receptor compartment were taken at different time points (0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8 hours) and an equal quantity of fresh PBS was replaced in the compartment after each withdrawal. The absorbance of each aliquot sample was measured at 420 nm using a plate reader and the concentration of BRN was determined using a standard calibration curve. Two independent experiments were carried out for triplicate readings**.**

The standard BRN curve was made and using the standard curve equation ($y = mx + c$), the amount of BRN released in the receptor compartment was calculated. To calculate the total amount of BRN released, the following formula was used.

Amount of BRN released (mg) = Concentration x Dilution factor x Volume of dissolution medium/1000

The % BRN release = Amount of BRN release (mg) x 100 /Dose (mg)

RESULTS AND DISCUSSION

The release rare in % of the BRN in unformulated BRN, conventional liposomal BRN and DELT- BRN are given in the Table 1. The release profile of all these samples at different time intervals are picturized in the Fig 1.

Table 1: Percentage of BRN release for the respective samples (Unformulated BRN, Conventional Liposomal BRN and DELT-BRN).

Time (hours)	Unformulated BRN (%)				Conventional Liposomal BRN (%)				DELT-BRN (%)			
	Set 1	Set 2	Set 3	Mean	Set 1	Set 2	Set 3	Mean	Set 1	Set 2	Set 3	Mean
0	0.06	-0.16	-0.24	-0.11	-0.28	-0.42	-0.34	-0.35	-0.22	-0.14	-0.22	-0.19
0.5	0.04	0.08	0.08	0.07	-0.04	-0.06	-0.08	-0.06	-0.02	-0.06	-0.08	-0.05
1	0.80	0.78	0.86	0.81	0.48	0.72	0.76	0.65	0.87	0.88	0.85	0.86
2	1.56	1.52	1.56	1.55	1.35	1.33	1.34	1.34	1.55	1.79	1.94	1.76

3	2.24	2.28	2.18	2.23	2.03	2.06	2.25	2.11	2.45	2.54	2.57	2.52
4	2.26	2.30	2.44	2.33	2.77	2.95	3.00	2.91	3.47	3.48	3.21	3.39
5	2.40	2.34	2.42	2.39	3.40	3.71	3.42	3.51	3.97	3.90	3.90	3.92
6	2.76	2.48	2.32	2.52	3.80	3.73	3.72	3.75	4.47	4.70	4.59	4.59
7	2.24	2.24	2.32	2.27	3.93	3.98	3.98	3.96	4.59	4.75	4.92	4.76
8	2.22	2.26	2.32	2.27	3.98	4.12	4.10	4.07	4.90	4.75	4.88	4.84

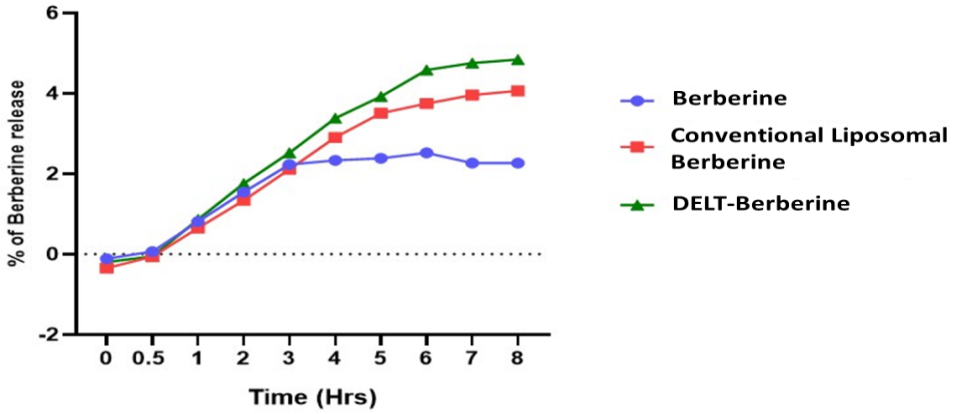

Figure 1: In vitro release profile of formulated BRN and unformulated BRN (%) at different intervals.

The Table 1 and Figure 1 show the release profile of BRN in the DELT and conventional liposomal formulations compared to the unformulated control, at pH 7.4. Owing to the poor solubility of BRN, the cumulative release of unformulated BRN and conventional liposomal BRN are less in comparison with advanced liposome (DELT-BRN), after the 3 hours it is quite evident. Interestingly, DELT-BRN showed a higher release trend from 3-8 h time points indicate the better stability due to the extra layer protection by the plant proteins. Unformulated BRN showed a least release (2.52%), then conventional liposomal BRN (4.07%) and DELT-BRN showed the highest release (4.84%) due to the better stability and solubility. DELT-BRN exhibited a significant release at 8 h, compared to the conventional BRN and control. The observed trend in the in vitro release profile of BRN clearly indicates that the DELT formulation enhances the solubility and stability of BRN in aqueous solutions and thus eventually leads to a higher bioavailability.

CONCLUSION

Both control, conventional and advanced liposomal (DELT) BRN exhibited comparable drug release effects for up to second hours. After four hours, the release profile of DELT-BRN increased significantly in comparison with control and conventional liposome, indicates the higher solubility and stability in aqueous solutions and thus eventually leads to a higher bioavailability.

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