The Pharma Innovation Journal 2021; 10(10): 2141-2146

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(10): 2141-2146

www.thepharmajournal.com
Received: 14-07-2021
Accepted: 29-09-2021

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In vitro digestion study of bioactives infused Oleogel

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Abstract
Oleogelation is a technique of structuring of oil, in the presence of gelator molecules. The oleogels formed have got superior functional benefits especially in replacing the harmful trans fats and other conventional shortenings in bakery and meat-based products. Apart from that due to their lipophilic nature, oleogels have been recently studied as nutraceutical carriers especially for hydrophobic bioactive compounds. The bioactives are generally very sensitive towards light, heat and harmful pH conditions that there is a continuous demand for developing an effective carrier. In this study the ethylcellulose oleogels, which are a well-accepted type of oleogels, utilizing its lipophilic medium was explored as nutraceutical carrier for co-delivering curcumin and resveratrol. It was observed that the oleogel containing 11% w/w of EC promoted a targeted co-delivery of both curcumin and resveratrol (> 50%) under intestine condition. This study expands the application of EC-MCT based oleogels as bioactive carriers in nutraceutical development.

Keywords: MCT oil, ethylcellulose, co-delivery, curcumin, resveratrol, nutraceutical

1. Introduction
In recent times, there is an increasing demand for alternate fat to replace unhealthy saturated fats and hydrogenated vegetable oils in food applications. The negative impacts the trans fats impose on human health have made serious concerns on its usage in food industry. Due to this U.S. FDA not only eliminated trans fats from GRAS (Generally regarded as safe) list in 2015 but also has completely banned usage of partially hydrogenated oils from January 2020 (Adili *et al*., 2020) [1]. This shift from conventional fats has increased the demand for alternate fats and has contributed to a significant number of attempts by researchers in developing such structured oil. In this regard, oleogel, structured oil has been proven effective in substituting to replacing a significant amount of conventional fat in food systems (Boukid, 2021; Dreher *et al*., 2021) [2, 8]. In case of food applications, solid fats express better functional benefits than liquid oils in modifying various parameters including texture, taste, and flavor profile of the food products (W. Zhao *et al*., 2021) [31].
Oleogelation is a technique involving the use of different gelators and subjecting them to mild to high temperatures to allow the network development such that the oil phase is trapped within the gelator networks (Gravelle *et al*., 2016) [12]. It is reported that the gelator networks can be formed by self-assembled structures or crystallization that yield a three-dimensional network entrapping the liquid oil. Among oleogelators, commonly used and only FDA approved polymer is the ethylcellulose which is the gelator used in this study. Among oleogels, ethylcellulose based oleogels are well explored and it is reported to structure oil through direct dispersion. The viscosity of EC polymer would dominate the overall mechanical properties of the final oleogel (Cabrera *et al*., 2020; Scharfe & Flöter, 2020) [3, 26]. In this study, medium chain triglycerides (MCT) oil was chosen as oil phase because of its higher health benefits (Lee *et al*., 2021) [14], but it is also reported that it cannot be taken as such due to their undesirable organoleptic properties and sometime its ability to cause digestive distress when consumed at huge amount. Hence when it's converted to oleogel it could be incorporated in various types of food products. Some of the examples of EC based oleogels included in food products include bakery products (Gómez-Estaca *et al*., 2019) [10] (M. Zhao *et al*., 2020) [30], chocolate (Espert *et al*., 2021; Li *et al*., 2021; Li & Liu, 2019) [9, 16, 15] also meat-based products (Moghtadaei *et al*., 2021; Oh *et al*., 2019) [21, 25].
As it is known that under several harsh conditions like light, acidic medium, temperature the sensitive bioactive compounds undergo degradation and on top of their poorly solubility makes it necessary to find a suitable medium to carry these compounds in the human system (Tang, 2021) [28]. In this view, lipid-based carriers are highly useful being carriers for bioactive

compounds like emulsion, nano-emulsion, solid lipid nanoparticles. In this regard, oleogels providing lipophilic environment have also been studied as nutraceutical carrier and few studies have been reported (O'Sullivan *et al*., 2016) [24]. In this study, two bioactive compounds curcumin and resveratrol were chosen because of their synergistic health effects especially in treating cancer patients. Among other combinations curcumin and resveratrol was proven effective against obesity, neurodegeneration and inflammation and especially for cancer treatments (Chen *et al*., 2017; Mukherjee *et al*., 2018; Shindikar *et al*., 2016) [4, 22, 27]. The nutraceutical containing two or more bioactive compounds or combination of two nutraceuticals could be the future that they are called the next generation medicines (Leena *et al*., 2020), This study deals with the development of EC based oleogel and its effectiveness as nutraceutical carrier for co-delivering curcumin and resveratrol.

2. Materials and Methods

Ethylcellulose (EC) with viscosity of 90-110 cP was obtained from TCI, Chemicals. Medium chain triglycerides (MCT) oil was procured from Luxura Business Pvt. Ltd, India. Enzymes like α-amylase, pepsin, bile salt, lipase, resveratrol and curcumin were obtained from Sigma Aldrich, Pvt., Ltd. India. All inorganic salts required during preparation of simulated digestion fluids were purchased of analytical grade from HiMedia Laboratories Pvt. Ltd, India

2.1. Oleogel Preparation

To prepare oleogel, the minimum EC required for gelating MCT oil was determined by varying its amount from 3-11% w/w. Briefly, the preparation involved heating the EC-oil mixture upto 180 ℃ in a magnetic stirrer at 300 rpm for complete dissolution and later it was cooled down to room temperature for gel setting.

2.2. Bioactive loading and encapsulation efficiency

To the optimised oleogel concentration, of curcumin and resveratrol each at 2.5% w/w with respect to the total oil percentage were added to the molten EC-oil mixture for complete dissolution and set quickly by bringing to room temperature to reduce the increased exposure to higher temperature. The bioactives were quanitified spectrophotometrically using SpectraMax® iD3 (Molecular Devices, LLC, USA).and loading capacity (LC) and encapsulation efficiency (EE) were found according to (Guo *et al*., 2021) [13]

$$,LC\% = \frac{Mass\ of\ entrapped\ nutraceutical}{Total\ mass\ of\ oleogel} \times 100 \text{(Eqn.1)}$$

$$EE\% = \frac{Entrapped\ nutraceutical\ in\ oleogel}{Total\ mass\ of\ nutraceutical\ added\ in\ oleogel} \times 100 \text{(Eqn.2)}$$

2.3. Oil binding capacity

The oil binding capacity was determined by (Adili *et al*., 2020) [1], according to which 0.5-1 ml of oleogel sample was taken in centrifuge tubes and kept for centrifugation at 10,000 rpm for 15 minutes. The weight before and after centrifugation for sample containing Eppendorff tubes were measured

$$.\%\ oil\ released = \frac{mass\ of\ released\ oil\ (g)}{Total\ mass\ of\ sample\ (g)} \times 100 \text{(Eqn.3)},$$

$$OBC = 100 - \%\ oil\ released \text{ (Eqn.4)}$$

2.4. Microscopic structure analysis

To understand the microstructure of oleogels, the oleogel sample was added on the glass slide and set for 24 hours. It was viewed under polarized light microscopy at 40X magnification in RTC-7NX series, Radical Scientific, India.

2.5. Texture Analyzer

To understand the hardness of oleogels, the samples were set in glass vials and gel strength was measured using texture profile analyzer, TA-HD Plus (Stable Micro Systems, Surrey, UK) using a spherical P/0.25S spherical probe. The penetration speed was set at 1 mm/s upto 5 mm depth. The samples were stored for 24 hours before analyzing (Davidovich-pinhas *et al*., 2015) [7, 8].

2.6. Bioaccessibility studies

For the optimised oleogel composition with added bioactive compounds, the release profile for both curcumin and resveratrol were studied under *invitro* digestion. The simulated digestion buffers were prepared according to Minekus *et al*., (2014) [20]. The method was referred from (Chloe *et al*., 2017) [5] according to which 0.5 g of sample was taken and cut into small pieces of diameter <1mm. Initially, for salivary phase of digestion 5 ml of SSF was taken containing 75 units of α-amylase and added to the sample and pH was maintained at 7 before incubating at 37 ℃ for 2 minutes. To initiate gastric digestion, SGF of 5 ml of was added to the bolus solution with pepsin maintained at 2000 units with respect to final mixture. The pH was brought down to 3 before incubating for 2 hours at 37 ℃. After 2 hours, 10 ml of SIF was added to the chyme solution with 2000 units of lipase and 10 mM of bile salts and the solution was maintained at pH 7 for next 2 hours of digestion. The bioaccessibility was found using LC and EE values obtained from Eqn.1 and 2 using Eqn.5 given by (Yu *et al*., 2012) [29],

$$Bioaccessibiity\% = \frac{Amount\ of\ solubilised\ bioactives}{Amount\ of\ bioactives\ present\ in\ sample} \times 100 \text{ (Eqn.5)}$$

2.7. Statistical Analysis

Results of triplicate measurements were reported as average ± standard deviation. Variance of the data was analyzed using SPSS 22.0 version statistical analysis software system. One-way ANOVA was used to compare mean values using 5% of level of significance for the bioaccessibility studies.

3. Results and Discussion

3.1. Optimization of oleogel development

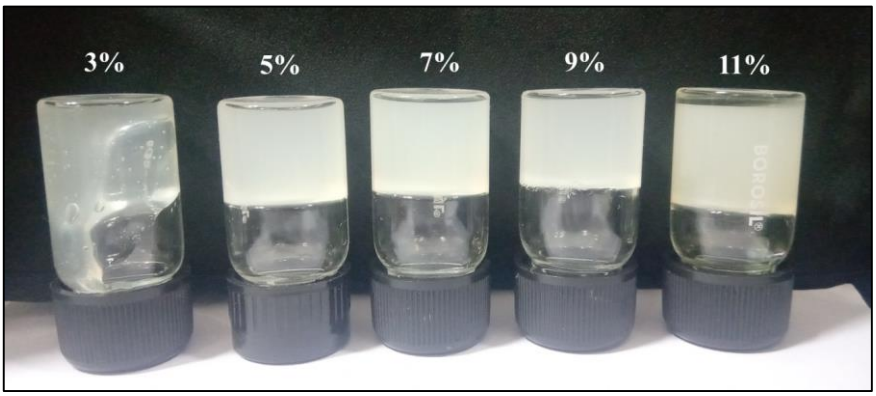

Fig 1: Pictorial representation of different EC concentration in oleogel

In this study, EC based MCT oleogel was developed by direct dispersion method and its ability to carry and co-deliver synergistic nutraceuticals, curcumin and resveratrol was studied. When the amount of EC was varied from 3-11% w/w in structuring MCT oil, it was found that minimum of 5% w/w was required for oleogelation (Fig.1). It could be observed that 3% w/w is not sufficient to form a stable oleogel. From 5-11%, based on the oil binding capacity of the oleogels 11% w/w of EC was fixed in the formulation. Hence the bioactives were incorporated in 11% w/w EC oleogel and the effectiveness of the oleogel as nutraceutical carrier was studied using *in vitro* digestion study.

3.2 Effect of oleo gelation on OBC

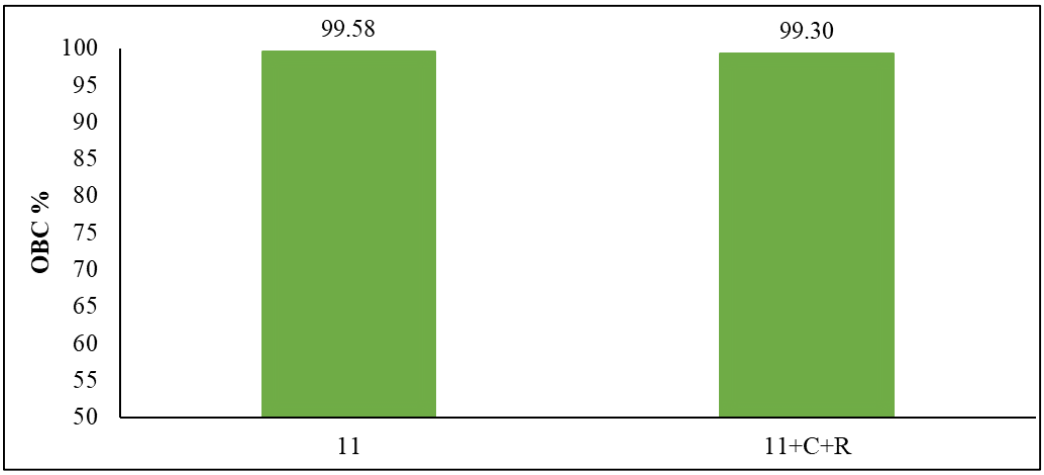

Fig 2: Oil binding capacity (OBC) for oleogels EC11 and EC11 (Cur+ Rsv) which indicates the bioactives incorporated oleogel

The OBC of oleogels were calculated according to the Eqn. 3 and 4 to determine the efficiency of gelation from the Fig.2 as the addition of EC increased the OBC also increased, indicating the ability to form better networking by the polymer. With highest OBC, 11% (w/w) was chosen for oleogel development for further bioactives-curcumin and resveratrol incorporation. It could be seen that after addition of bioactives, there was no significant change in OBC of oleogel.

The bioactive molecules interfered in the hydrogen bonding between EC and MCT oil as the bioactives are completely dissolved in the oil phase. Thus, the curcumin and resveratrol compounds were speculated to influence the network formation (Gómez-estaca *et al.*, 2020) [11]

3.3 Microstructure analysis of oleogel

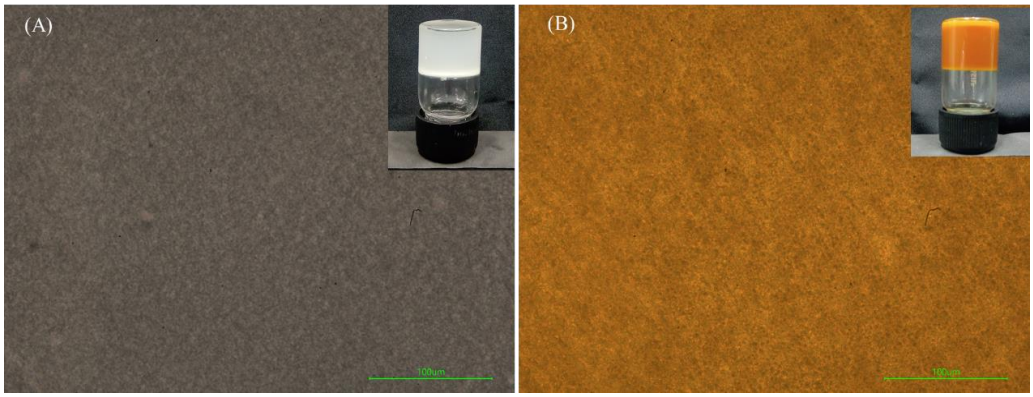

Fig 3: Polarized light microscopic view of EC oleogel (A) and curcumin and resveratrol infused EC oleogel (B) at 40x magnification. Scale-100 µm

To understand the influence of curcumin and resveratrol on oleogel structure, microscopic view was observed under polarized light microscopy which indicated the uniform structuring of oil phase (Fig. 3A). After bioactives inclusion, it was observed that there was a uniform dispersion in the oleogel network. Also, in Fig.3B, there were no crystals observed which could be due to complete dissolution of curcumin and resveratrol. Similar results were observed by Liu *et al*., (2020) [17] where uniform dissolution was curcumin was observed in EC oleogel. This is because the bioactives were added at the hot molten state of oleogel at which they will undergo quick dissolution. Hence these results prove that the bioactives are uniformly and completely solubilized in the oleogel matrix.

3.4 Textural analysis of oleogel

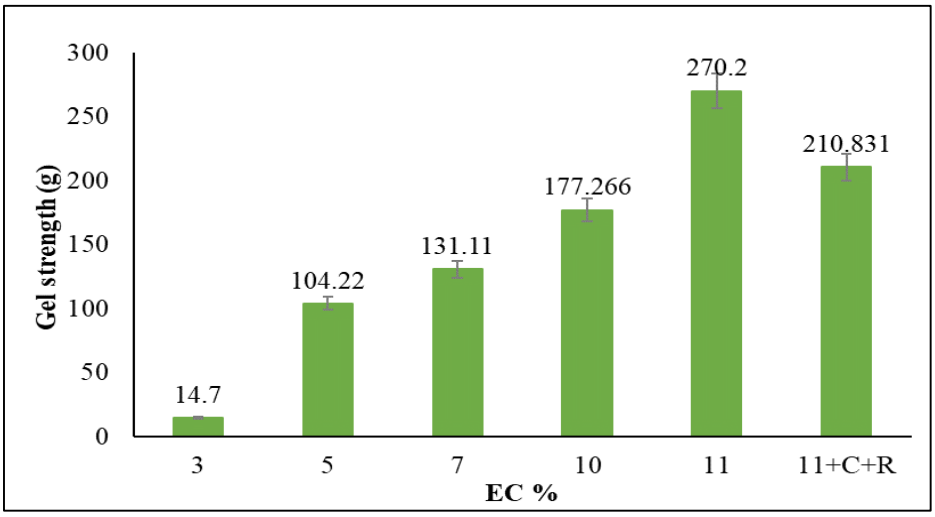

Fig 4: Gel strength of oleogel

In general, the ethylcellulose oleogels of 100 cP exhibited higher gel strength than 20 cP and 45 cP irrespective of the type of oil used (Maya Davidovich-pinhas *et al*., 2015) [7, 8]. With the increase in addition of EC the network strength was found to increase, as observed in Fig.4. This is due to the increase in hydrogen bonding among EC polymer molecules that attributed to the increase in bonding junctions (Masotta *et al*., 2019) [18]. But after bioactives addition there was a reduction in strength which indicate that they have interfered in the network formation, which was also evident in OBC study. Due to this there was a reduction in mechanical strength of oleogels.

3.5 Encapsulation properties: To calculate EE, known amount of sample, around 13-15 mg, was taken and added to 10 ml of water and vortexed for 10 minutes. Then 70% methanol was added to the above sample and vortexed for 2 h. It is done to ensure complete disintegration of oleogel matrix so that all the bioactives are released (Chloe *et al*., 2017) [5]. The encapsulation properties of oleogel were found according to Eqn. 1 & 2. According to the formulation, LC was found to be 2.02% each for curcumin and resveratrol in the oleogel and EE was found to be 80.55 ± 2.48% and 91.17 ± 1.38% for curcumin and resveratrol respectively.

3.6 *In vitro* digestion release profile

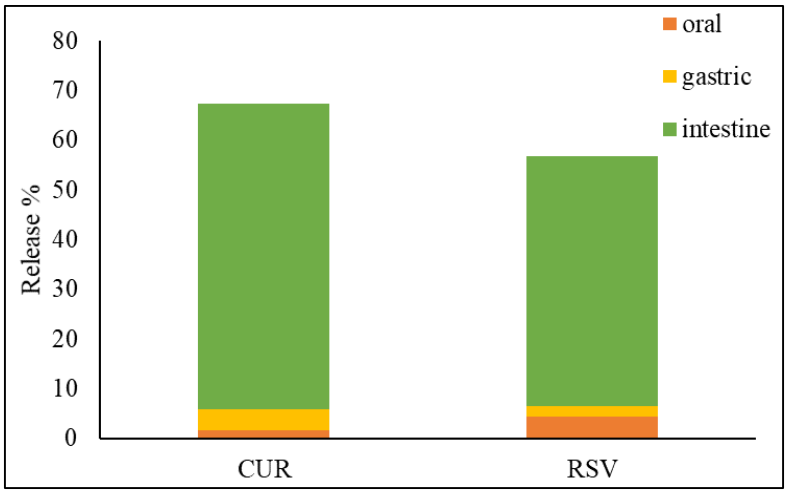

Fig 5: *In vitro* release profile for curcumin and resveratrol in EC oleogel under simulated digestion studies

To evaluate the release characteristics of curcumin and resveratrol, the bioactives loaded oleogel was subjected to *in vitro* simulated digestion studies using simulated salivary fluid (SSF), simulated gastric fluid (SGF) and simulated intestine fluid (SIF) at corresponding pH levels. It could be seen in Fig.5 that the developed oleogel protect the loaded bioactives from oral and gastric conditions and promoted targeted release in intestine. The EC networks entrapping

bioactives in lipid medium protected the bioactives from adverse gastric condition. As it is previously reported that EC because of their nature could be used for colon-specific delivery (Amison *et al*., 2015) [3]. There is a strong relation between the type of network and the entrapped bioactives in gel type foods (Mulet-Cabero *et al*., 2017) [23]. In SSF, very less release has been obtained for both curcumin and resveratrol (<5%). This indicates that they are protected against salivary conditions. In SGF condition, only <10% of release was found which could be considered as a success as most of the degradation of bioactives occur at harmful stomach condition (McClements, 2018) [19]. In case of intestine conditions, it could be seen that majority of loaded bioactives were released under SIF condition, around 61.3% and 50.2% for curcumin and resveratrol, respectively. It was reported that the bioactives should get released from its matrix and involve in micelle formation for bioaccessibility, such that it gets absorbed through intestinal layer for systemic circulation (Zhou *et al*., 2021) [32]. Hence it can be seen that the developed EC based MCT oleogel supported enhanced targeted co-delivery for curcumin and resveratrol and has the potential to act as a nutraceutical carrier.

4. Conclusions

In order to protect the sensitive bioactive compounds like curcumin and resveratrol, a lipid-based carrier, oleogel was used as used to evaluate its ability as nutraceutical carrier. As a commonly used technique *in vitro* digestion study was used in this study for the objective. From the results, it was found that EC-MCT based oleogel promoted a targeted intestine co-delivery such that around 61% and 50% of release were found for curcumin and resveratrol respectively. Hence it could be concluded that an effective nutraceutical carrier for co-delivery of curcumin and resveratrol was developed. Because of their unique composition they could be easily incorporated in any food systems in turn expanding its application.

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