Journal of Drug Delivery Science and Technology 112 (2025) 107286

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst

Green techniques to develop food-grade resveratrol Eudraguard® Biotic/control microparticles as a shield against stress conditions

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ARTICLE INFO

Keywords:
Food supplements
Nutraceuticals
Intestinal delivery
Biopolymers

ABSTRACT

Inflammatory bowel diseases (IBD) are a growing global concern, with current treatments—primarily anti-inflammatory drugs and biologics—facing limitations such as side effects, high cost, resistance, and low patient compliance.

This study proposes a nutraceutical-based alternative by developing food-grade Eudraguard® Biotic/Control microparticles (MPs) encapsulating trans-resveratrol (RSV), the isoform with superior anti-inflammatory activity. Two green encapsulation methods, emulsion solvent evaporation (ESE) and solvent evaporation (SE), were employed to protect RSV from degradation and achieve sustained colonic release. Solid-state characterization and in vitro studies assessed stability (under UV, light, heat, and long-term storage), dissolution behavior, and release kinetics. Both techniques effectively preserved RSV's trans-isomer and minimized premature release in the gastric and intestinal phases, enabling targeted and prolonged delivery (up to 24 h) in the colon. Further in vitro and in vivo investigations will assess the safety and therapeutic efficacy of these systems.

1. Introduction

Inflammatory bowel diseases (IBD), including ulcerative colitis and Crohn's disease, are a growing global health concern. While incidence rates remain stable in Europe and North America, they are increasing rapidly in newly industrialized regions such as Asia, Africa, and South America [1]. Recent advances have identified several factors contributing to IBD onset, including genetic predisposition, environmental triggers (e.g., diet, psychological stress, sleep deprivation), and microbial dysbiosis [2].

The primary goal of IBD therapy is to control chronic inflammation, maintain remission, and prevent disease flare-ups [3]. Conventional pharmacological treatments—such as immunosuppressants, aminosalicylates, and corticosteroids—can be effective but are often associated with significant side effects, including vomiting, diarrhea, lymphopenia, liver enzyme alterations, and pancreatitis [4]. Biologic therapies (e.g., infliximab, adalimumab, vedolizumab) have emerged more recently [5–7], but their use is limited by high cost, parenteral administration, and a substantial number of non-responders [8]. To overcome these limitations, this study proposes a nutraceutical-based therapeutic strategy using resveratrol (RSV), a natural polyphenolic compound with potent antioxidant and anti-inflammatory activity [9–14]. However, RSV's clinical application is hindered by low bioavailability, due to poor water solubility, rapid degradation, presystemic metabolism, and isomerization from the therapeutically active trans-isomer to the less effective cis-form [9,15–17]. This isomerization is triggered by exposure to light, heat, and UV radiation, making stabilization of the trans-RSV essential to maintain its therapeutic potential [18,19].

Encapsulation of resveratrol (RSV) into particulate systems represents a promising strategy to protect the compound from environmental degradation and to deliver the active therapeutic form (trans-RSV) to its site of action [20,21]. In this study, microparticle (MP) systems suitable for oral administration were developed to enable RSV delivery along the

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https://doi.org/10.1016/j.jddst.2025.107286
Received 24 December 2024; Received in revised form 27 June 2025; Accepted 14 July 2025
Available online 16 July 2025
1773-2247/

gastrointestinal tract (GIT), with a targeted and sustained release in the colon. Oral formulations are preferred due to their advantages in terms of patient compliance, minimal invasiveness, safety, ease of administration, and therapeutic efficacy [22–25]. However, compounds administered orally must withstand gastric acidity and enzymatic degradation in the intestine, which can compromise their stability and efficacy [26].

Encapsulation of RSV in MPs acts as a protective shield, limiting exposure to harsh conditions in the upper GIT and promoting release at the target site. The primary aim of this study was to develop food-grade polymeric MPs capable of protecting RSV from environmental degradation while ensuring a controlled and prolonged release in the colon. To this end, MPs based on Eudraguard® Biotic (EUG-B) and Control (EUG-C) were formulated using green, solvent-free methods. These pH-sensitive copolymers, approved as food-grade additives, allow selective release of active substances in response to intestinal pH changes, with Biotic being responsive at pH 7.4 (colon) and Control at pH 6.8 (small intestine).

Biotic and Control polymers were used either individually or in combination—at ratios of 90:10 (w/w) and 70:30 (w/w) for both the emulsion solvent evaporation (ESE) and solvent evaporation (SE) techniques. Both approaches employed eco-friendly processes. Comprehensive solid-state physicochemical characterisation, stability under stress conditions, and in vitro release studies were performed to assess the systems' performance and functionality.

2. Materials and methods

2.1. Materials

Polymeric MPs were prepared using Eudraguard® Biotic (EUB) and Control (EUC) as polymer matrix, manufactured by Evonik Nutrition & Care GmbH (Darmstadt, Germany) and kindly provided by Rofarma Italia S.r.l. (Gaggiano, Italy). Trans-resveratrol (T-RSV) was kindly donated by Labomar S.r.l. (Istrana, Italy). Span® 80 (sorbitan monooleate) and acetone were purchased from Merck (Darmstadt, Germany). Paraffin oil was purchased from Farmalabor Srl (Canosa di Puglia, Italy). Aerosil® 200F is produced by Evonik Nutrition & Care GmbH (Darmstadt, Germany) and kindly provided by Rofarma Italia S.r.l. (Gaggiano, Italy). Petroleum ether and ethanol were purchased from Merck Italia spa (Milan, Italy). HPLC-grade acetonitrile (VWR Chemicals) was purchased from VWR International OY (Helsinki, Finland).

2.2. Dialysis purification of Edudraguard® polymers

Commercially available Eudraguard® Biotic (EUG-B) and Control (EUG-C) are 37 % (w/v) aqueous dispersions with different concentrations of surfactants as emulsifying agents, specifically polyethylene glycol monostearyl ether in EUG-C and sodium lauryl sulfate and polysorbate 80 in EUG-B [27]. To get rid of these undesirable substances, the dialysis purification method previously reported was used [28]. The polymer suspensions were dialyzed for 24 h using a semi-permeable membrane (Spectra/Por porous membrane, MWCO: 3.5 KD. The membranes were submerged in 2 L of double-distilled (d.d.) water under magnetic stirring at room temperature for 24 h. The medium was changed every 2 h to remove surfactants. Then, the resulting dialysate was frozen at −20 °C and subjected to lyophilisation using the Buchi Lyovapor L-200 apparatus at a temperature of −57 °C and a pressure of 0.800 mbar.

2.3. Preparation of polymeric MPs by emulsion solvent evaporation (ESE) and solvent evaporation (SE)

Polymeric MPs were obtained using two techniques previously reported [29]: Emulsion Solvent Evaporation (ESE) and Solvent Evaporation (SE).

2.3.1. Emulsion solvent evaporation (ESE)

In the ESE technique, the acetone solution (20 mL), constituted by 250 mg of EUG-B and 250 mg of EUG-C (and 20 mg of RSV for RSV loaded MPs), was dripped into 25 mL of paraffin oil containing 1 % (w/v) Span® 80 under magnetic stirring (500 rpm) in an ice bath for 30 min. Then, it was evaporated overnight at room temperature. Therefore, 100 mL of petroleum ether was added to the mixture under magnetic stirring (500 rpm) for 1 h to disaggregate the MPs. Subsequently, the mixture was thoroughly decanted. The collected MPs were repeatedly washed with petroleum ether and filtered under vacuum through a Hirsch funnel. Finally, the resulting powder was dried under vacuum at 40 °C for 24 h in a Büchi glass oven.

Hollow MPs were produced (ESE.0.B, ESE.0.C, ESE.0.90B and ESE.0.70B) and loaded with RSV in a polymer to RSV weight ratio of 1:1 (ESE.R.B, ESE.R.C, ESE.R.90B and ESE.R.70B).

2.3.2. Solvent evaporation (SE)

In the SE technique, unlike the ESE technique, no dialysis purification step was required for the commercial suspensions of EUG-B and EUGC-C. In fact, the commercial polymer suspensions were directly subjected to lyophilisation following the procedure described above (2.2.). Thus, the polymeric systems were prepared with an m\m ratio between RSV and polymer of 1:10. Specifically, the organic phase consisted of ethanol (25 mL) in which 250 mg of EUG-B and 250 mg of EUG-C (and 20 mg of RSV for RSV-loaded MPs) were solubilised under magnetic stirring at a temperature between 37 and 40 °C. The organic phase was dripped into an equivalent amount of water d.d. The organic solvent was evaporated by rotaevaporation to promote polymer precipitation and MPs formation [30–32]. Finally, the suspension obtained was frozen at −20 °C and lyophilised following the procedure previously described (2.2.).

Preliminary studies revealed the presence of aggregates in the systems after the freeze-drying process. To overcome this issue, Aereosil® 200F, a modified silicate approved for food use [33], was introduced before the freeze-drying step. In MPs systems consisting of both polymers EUG-B and EUG-C, 250 mg of silicate suspension was added; in contrast, in MPs systems consisting of EUG-C only, 100 mg of silicate suspension was added.

2.4. FT-IR spectroscopy analysis

A Fourier transform spectrophotometer (1600 FT-IR spectrophotometer; PerkinElmer Italia SpA, Milan, Italy) was used to conduct IR analysis of the following samples: unloaded MPs (ESE.0.B, ESE.0.C, SE.0.B and SE.0.C) and loaded MPs (ESE.R.B, ESE.R.C, SE.R.B and SE.R.C) at zero time (T0) and at 24 months storage (T24) at 25 ± 2 °C/RH = 60 ± 5 %; EUG-B; EUG-C; Aerosil® 200F; physical mixture (EUG-B and EUG-C 1:1) 1 mg aliquots of loaded MPs at T0 and T24 destroyed in methanol (30 min under magnetic stirring) and dried. The solid samples were mixed with anhydrous KBr in a ratio of 1:10. Subsequently, the solid mixture was compressed into into 1-mm disks and the background was acquired from a pure KBr disk. The instrument was provided with an attenuated total reflectance (ATR) attachment and a diamond/zinc selenide (diamond/ZnSe) crystal window. The results were derived from 20 scans acquired in the range of 400–4000 cm-1 with a resolution of 2 cm-1 at room temperature.

2.5. High-performance liquid chromatography (HPLC) methods for the quantification of trans-RSV and cis-RSV

HPLC analysis was performed, modifying the method of Flieger et al. [34] and using an Agilent 1100 binary pump (Agilent Technologies Inc., Wilmington, DE, USA), a 1100 micro vacuum degasser, a HP 1050 Autosampler, a HP 1050 variable wavelength detector (operated at 235 nm). The chromatographic separations were achieved on a ZORBAX Eclipse XDB-C18 (2.1 mm × 100 mm, 1.8 μm) (Agilent, USA) by using

isocratic elution of Milli-Q® water and ACN (75:25, v/v). Effluent was monitored at a wavelength of 310 nm and 220 nm, with a flow rate of 0.3 mL/min; the injection volume was 5 μL; retention time of 5.2 min for trans-RSV and 9.1 min for cis-RSV. The column was maintained at 45 °C throughout the analysis.

RSV standard calibration curves was prepared in Milli-Q® water/EtOH (20 % v/v) with a linear regression coefficient determined in the range 0.05–100 μg/mL for trans-RSV and 0.1–100 μg/mL for cis-RSV. The linear regression coefficients were 0.9999 and 1 respectively.

The experiment was done in triplicate and the HPLC-UV analyses were performed in duplicate. The results are reported as mean ± standard deviation.

2.6. Drug loading (% DL) and Encapsulation Efficiency (% EE) evaluation of polymeric MPs

To evaluate the % DL and % EE of the dried MPs, RSV was extracted dissolving dried MPs by vortexing for 5 min in the Acetone (0.5 mg/mL). The organic solvent was totally evaporated overnight under vacuum at room temperature. In order to form a solution only with the drug, 700 μL of Milli-Q® water/EtOH (20 % v/v) was added in each sample and they were vortexed for 2 min. Then, every sample was centrifugated at 10000×*g* at 4 °C for 30 min, to remove the polymeric residue, and the supernatants were withdrawn and injected into the HPLC-UV. The samples were analysed by HPLC-UV according to the method described in paragraph 2.5.

% DL was calculated according to the following formula:

$$\%\ DL = \frac{Experimental\ drug\ amount\ (mg)}{Total\ formulation\ amount\ (mg)} \times 100$$

% EE was calculated according to the following formula:

$$\%\ EE = \frac{Experimental\ drug\ amount\ (mg)}{Theoretical\ drug\ amount\ (mg)} \times 100$$

The experiments were repeated in duplicate, and the results are reported as mean ± standard deviation.

2.7. In vitro studies

2.7.1. Stability studies of dried MPs

Stability studies were conducted to assess whether polymeric systems, formulated with both SE and ESE techniques, are able to protect the encapsulated drug from the changing conditions of the external environment. Considering that RSV has been shown to be unstable when exposed to light, rapidly switching from trans to cis conformation [33], the dry MPs were exposed to the following conditions.

- tightly closed amber container stored for 24 months (25 ± 2 °C/RH = 60 ± 5 %);
- light at room temperature for 7 days (25 ± 2 °C/RH = 60 ± 5 %);
- climatic chamber (GmbH, Tuttlingen, Germany) for 7 days (40 ± 2 °C/RH = 75 ± 5 %);
- UV light for 6 h (25 ± 2 °C/RH = 60 ± 5 %);

The experiment was conducted in duplicate and the results are reported as mean ± standard deviation. The drug was extracted in each sample (500 mg) according to the method described in paragraph 2.6.; they were analysed by HPLC-UV using the method reported in paragraph 2.5.

2.7.2. RSV dissolution assay

A 'Dissolution paddle tester' device for solid pharmaceutical forms (ERWEKA DZT GmbH, Langen, Germany) was used to determine the dissolution rate of RSV, at different pH levels and under sinking conditions, following the protocol of studies carried out previously [35]. The physiological conditions of the gastrointestinal tract (GIT) can be replicated in vitro by this apparatus. The test was carried out at 37 ± 0.5 °C and 100 rpm. Initially, the amount of drug to be used in each experiment was calculated in order to comply with the sink conditions. The drug sample (2,5 g) was poured into a gelatine capsule and immersed in the suitable dissolution medium. The three different GIT tract habitats were replicated under the appropriate pH conditions using: simulated gastric fluid (SGF, pH 1.2) for 2 h, simulated intestinal fluid (SIF) at pH 6.8 for 4 h and phosphate buffered solution at pH 7.4 for up to 24 h. A 0.1 N HCl solution (375 mL) was added to the dissolver beaker (SGF, pH 1.2) to replicate the stomach's acidic environment. After 2 h, the pH was raised to 6.8 (small intestine value) by adding 125 mL of a 0.2 M tribasic sodium phosphate solution that had been preheated to 37 ± 0.5 °C. A few drops of 2 N sodium hydroxide solution were added after another 4 h to get the pH up to 7.4 (colon value).

The same volume of the appropriate dissolving liquid was added to the test vessel to restore the initial volume after 2 ml aliquots were removed at regular intervals throughout the 24-h test period.

After centrifuging the samples for 30 min at 10,000 rpm and 10 °C, the clear supernatant was examined using UV spectrophotometry. The absorbance was compared to a calibration curve of RSV in the two reference solutions (0.1 N HCl and phosphate buffer pH 6.8), which were both linear in the range of 0–20 μg/mL ($R^2 = 0.9998$), and the corresponding concentrations were determined. The results are shown as mean ± standard deviation, and the experiment was conducted twice.

2.7.3. Release study of RSV from polymeric MPs

By investigating the release over time and in different simulated environments of the amount of RSV released by the polymeric MPs (release profile), it is possible to assess both the ability of the carrier to ensure delayed release at the colonic level (target site).

Following the procedure described above for the dissolution experiment, pH-dependent release tests were performed on the formulations loaded with 2,5 g of RSV (T0 and T24) derived from the production techniques ESE and SE. Every sample was examined either in triplicate or duplicate.

pH predetermined intervals, two-mL aliquots were taken out and replaced with the same volume of either 0.1 N HCl or phosphate buffer solution (pH 6.8 and 7.4, respectively). To determine the RSV concentration, the collected samples were centrifuged for 30 min at 10,000 rpm and 10 °C. The clear supernatant was then examined using a UV spectrophotometer.

2.7.4. Release kinetic analysis

Several kinetic models were used to analyze the polymeric MPs in vitro release data:

Model of zero order: $R = K_o t$

Model of first order: $R = 1\text{-}e^{-kt}$

Model Higuchi: $R = K_H\ t^{1/2}$

Model of Hixson-Crawell: $W_o^{1/3} - W_t^{1/3} = K_{HC} t$

Korsmeyer-Peppas model: $R = k_{KP}\ t^n$

t is the amount of RSV released at a given time. R is the expression for the constant k; in particular, the rate constants for zero-order, first-order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas kinetic models are k_o, k, k_H, K_{HC}, and k_{KP}, respectively. In the Korsmeyer-Peppas model, n is the release exponent; W_o is the starting drug quantity and W_t is the drug amount at time t [31,32]. To explain and assess the mechanism of RSV release, the model with the highest correlation coefficient (R^2) was chosen. The linear portion of the release curves (from 3 h to 6 h in SIF and from 7 h to 24 h in SCF forward) was calculated.

2.8. Statistical analysis

The data are expressed as mean ± standard deviation. Statistical analysis was performed using one-way ANOVA via StatPlus® software.

Table 1
EUG-B and EUG-C MPs loaded with RSV and formulated via the ESE and SE technique.

SAMPLES	EUG-B[a]	EUG-C[b]
ESE(SE).R.B	100	–
ESE(SE).R.C	–	100
ESE(SE).R.90B	90	10
ESE(SE).R.70B	70	30

[a] % w/w EUG-B/matrix.
[b] % w/w EUG-C/matrix.

3. Results

3.1. Macroscopic aspect of polymeric MPs

Eudraguard® resins are methacrylate copolymers approved as *food grade* by the European Commission and are commonly used as coating materials for solid oral nutraceuticals with modified-release profiles [4,28]. They provide key advantages such as gastric resistance, masking of unpleasant odors and tastes, and protection from moisture [29]. The Eudraguard® product line includes four pH-sensitive copolymers: Natural, Protect, Biotic, and Control [4,28].

Biotic (E1207) [36], an anionic copolymer composed of methyl acrylate, methyl methacrylate, and methacrylic acid (7:3:1), protects active compounds from stomach acidity and promotes release in the colon at pH 7.4 [4,29,35,37]. Control, a neutral copolymer of methyl methacrylate and ethyl acrylate (2:1), is GRAS-certified and EFSA-approved as a food additive. It enables sustained release in the small intestine by responding to pH 6.8 [4,29,35,37]. Both Biotic and Control are pH-sensitive, responding at pH $\geq$ 6.8 due to their functional groups [38,39].

Using the Emulsion-Solvent Evaporation (ESE) and Solvent Evaporation (SE) techniques, microparticles (MPs) were formulated with predetermined amounts of Eudraguard® Biotic (EUG-B) and Control (EUG-C), maintaining a polymer matrix to RSV weight ratio of 1:10 (Table 1). During SE preformulation, the formation of aggregates was observed; this issue was addressed by incorporating the inert stabilizer Aerosil® 200F (A), resulting in a matrix:A ratio of 1:1 for the SE.R.C system and 2:1 for the remaining formulations.

The polymeric systems prepared with the ESE technique show, under initial conditions (T0), characteristics overlapping with those described in the Curcio et al. [29] and Rizzo et al. [37] studies. In particular, the polymeric system containing 100 % EUG-B (ESE.0(R).B) presents itself as a homogeneous powder, whereas the system with 100 % EUG-C (ESE.0(R).B) as gummy flakes. The polymeric mixed systems (SE.0 (R).90B and ESE.0(R).70B) presented an intermediate appearance with increasing gummy appearance as the EUG-C concentration increased. Interestingly, none of the batches produced with this methodology showed signs of aggregation of significant particle size. All batches also showed a whitish colouration and were odourless.

Polymeric systems obtained using the SE technique have an amber colour. The powders obtained are odourless and low in density, as they are obtained after freeze-drying; they are also highly hygroscopic, so handling and storage are required to be done away from moisture. It is important to note that no batch produced by this method showed signs of aggregation of particles of considerable size. Furthermore, in the powder containing a higher concentration of EUG-C, a slight viscosity

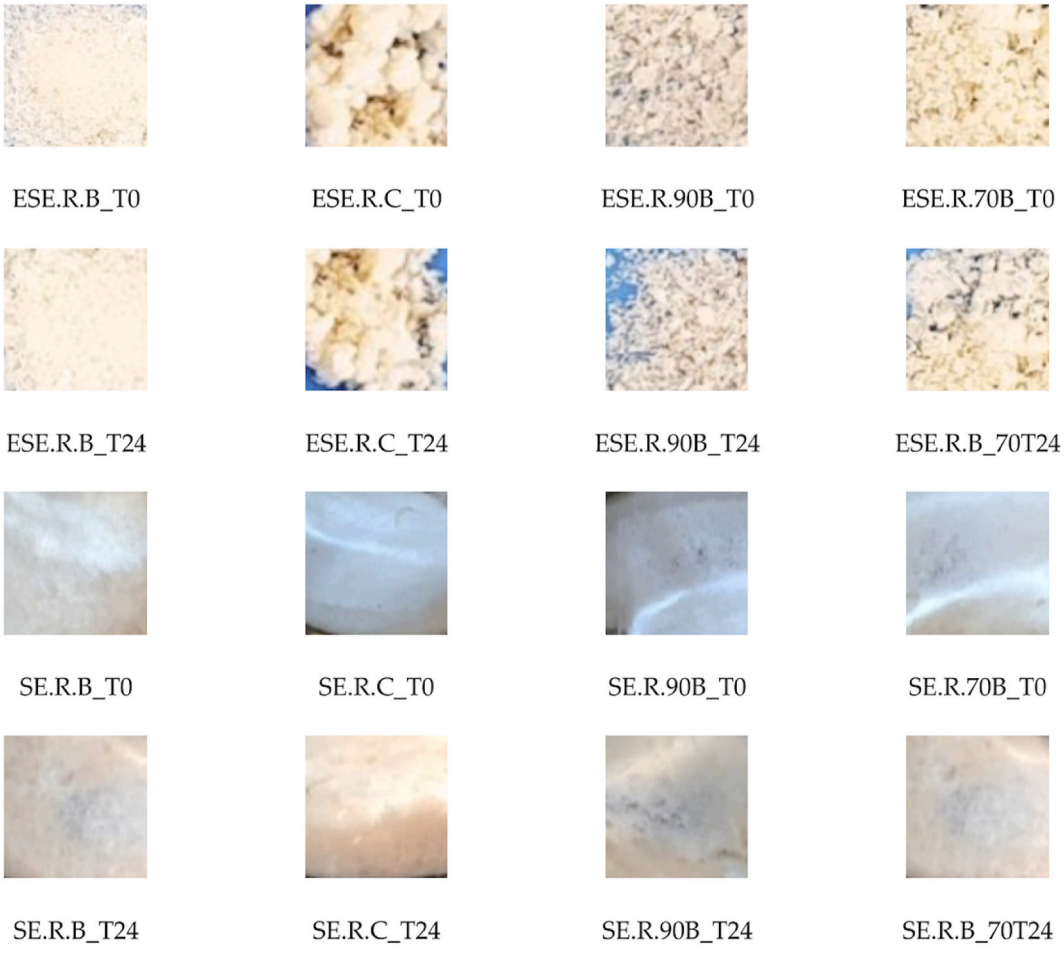

Fig. 1. MPs produced by the ESE and SE technique loaded with RSV at time zero (T0) and after storage for 24 months (T24).

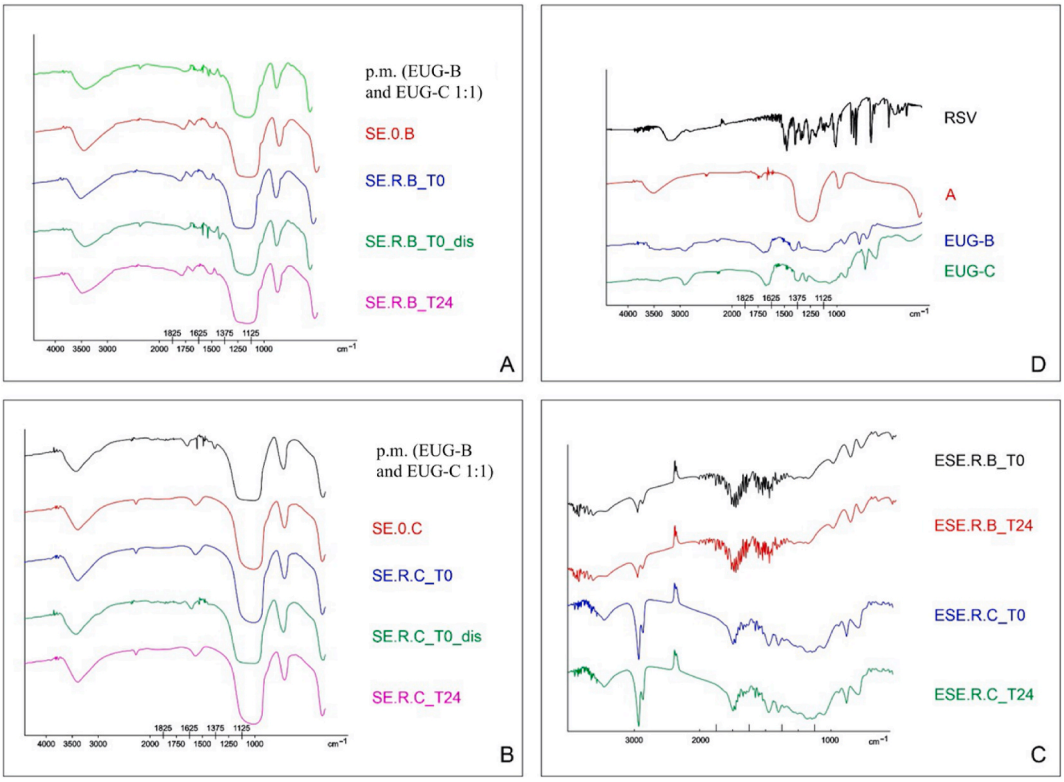

Fig. 2. FT-IR spectrum **A**: p.m. (EUG-B and EUG-C 1:1); SE.0.B; SE.R.B_T0; SE.R.B_T0_dis; SE.R.B_T24. FT-IR spectrum **B**: p.m. (EUG-B and EUG-C 1:1); SE.0.C; SE.R.C_T0; SE.R.C_T0_dis; SE.R.C_T24. FT-IR spectrum **C**: ESE.R.B_T0; ESE.R.B_T24; ESE.R.C_T0; ESE.R.C_T24. FT-IR spectrum **D**: RSV; A; EUG-B; EUG-C.

was observed.

Visual examination conducted on the loaded samples after storage in amber containers (25 ± 2 °C/RH = 60 ± 5 %) for 24 months did not reveal any significant changes. The powders were substantially unchanged in colour and odour.

3.2. Infrared analysis of polymeric MPs

To analyze the interaction and compatibility between the RSV and the polymer matrix and between the different materials used, FT-IR analysis was performed (Fig. 1 and Fig. 2). The FT-IR spectrum of the pure RSV exhibits the characteristic absorption bands at '3245.6', '1606.5', '1586.1', '1385.4' and '965.5' cm^{-1} which indicate the presence of 'OH', aromatic 'C=C', 'CC', 'CO' and trans-olefinic elongation vibrations, respectively, corresponding to the standard RSV [40]. The spectra of the unloaded MPs (ESE.0.B, ESE.0.C, SE.0.B and SE.0.C) and the corresponding physical mixtures (p. m.) are superimposable, which allows the hypothesis that the formulation processes involve neither alteration of the constituents nor the formation of new bonds between them. Furthermore, a comparison of the spectra of the p. m. and those of the corresponding constituents reveals that the former overlap with the latter. The spectra of the unloaded MPs are, moreover, superimposable with those of the respective loaded MPs (ESE.R.B_T0, ESE.R.C_T0, SE.R.B_T0 and SE.R.C_T0), proving that RSV has been encapsulated within the matrix. Indeed, no RSV-attributable peaks are found in the loaded MPs. Confirming this, the characteristic RSV bands are, on the other hand, identifiable in the spectra of the destroyed systems (SE.R.B_T0_dis and SE.R.C_T0_dis), highlighting the release of RSV after the destruction process of the MPs conducted in methanol and subsequent drying. The analysis carried out on the samples stored for 24 months (ESE.R.B_T24, ESE.R.C_T24, SE.R.B_T24 and SE.R.C_T24) confirmed the absence of alterations found on a macroscopic scale on visual analysis. The spectra of the systems loaded after storage are overlapping with those of the corresponding ones at T0, showing the absence of RSV segregation phenomena and constituent alterations.

Table 2
%DL and %EE of MPs loaded with RSV and prepared by ESE and SE techniques.

SAMPLES	%DL	SD	%EE	SD
ESE.R.B	3,10782487	0,01938102	80,8034466	0,50390653
ESE.R.C	2,41148697	0,00790638	62,6986612	0,20556577
ESE.R.90B	2,85255424	0,14352512	74,1664103	3,73165299
ESE.R.70B	2,51797405	0,32957896	71,5265607	8,56905288
SE.R.B	3,61612807	0,79548628	94,0193298	2,6826434
SE.R.C	2,96682752	0,93465217	77,1375154	2,3009564
SE.R.90B	3,76546157	0,09711123	97,9020009	2,52489195
SE.R.70B	3,67823788	0,54823541	94,8170925	1,15554329

3.3. Drug loading (% DL) and Encapsulation Efficiency (% EE)

The ESE and SE systems were analysed by HPLC analysis (paragraph 2.5.) to assess their 'Drug Loading' (%DL) and 'Encapsulation Efficiency' (%EE) (Table 2). The efficiency of systems prepared by the ESE technique ranges from 63 % to about 81 %. All systems showed good encapsulation of RSV within the MPs, the best results were obtained with the systems prepared with high concentrations of EUG-B (ESE.R.B and ESE.R.90B).

The efficiency of the systems prepared with the SE technique ranges from approx. 77 %–97 %. Again, a good encapsulation of RSV within the MPs is observed, and once again EUG-B also performed better than the systems prepared with the ESE technique.

3.4. Stability studies of dried MPs

As widely discussed above, RSV is a nutraceutical of great interest due to its extraordinary antioxidant capabilities. However, due to its

Table 3
Percentages of trans-RSV and cis-RSV extracted from polymeric systems produced by the ESE and SE technique at following conditions: T0; tightly closed amber container stored for 24 months (25 ± 2 °C/RH = 60 ± 5 %) (T24); light at room temperature for 7 days (25 ± 2 °C/RH = 60 ± 5 %); climatic chamber for 7 days (40 ± 2 °C/RH = 75 ± 5 %); UV light for 6 h (25 ± 2 °C/RH = 60 ± 5 %).

SAMPLES	T0		T24		Light (25 °C - 7 days)		40 ± 2 °C (7 days)		UV (25°C-6 h)	
	%TRANS ± SD	%CIS ± SD	%TRANS ± SD	%CIS ± SD	%TRANS ± SD	%CIS ± SD	%TRANS ± SD	%CIS ± SD	%TRANS ± SD	%CIS ± SD
ESE.R.B	100 ± 0	0	100 ± 0	0	97.422 ± 0.013	2.578 ± 0.013	99.572 ± 0.027	0.428 ± 0.027	91.291 ± 6.723	8.709 ± 6.723
ESE.R.C	100 ± 0	0	100 ± 0	0	93.891 ± 1.688	6.109 ± 1.688	94.923 ± 1.366	5.077 ± 1.366	95.641 ± 1.376	4.359 ± 1.376
ESE.R.90B	100 ± 0	0	100 ± 0	0	98.358 ± 0.118	1.642 ± 0.118	98.898 ± 0.676	1.102 ± 0.676	92.440 ± 0.186	7.560 ± 0.186
ESE.R.70B	98.259 ± 0.051	1.741 ± 0.051	98.260 ± 0.051	1.741 ± 0.051	98.027 ± 0.025	1.973 ± 0.025	98.510 ± 1.260	1.490 ± 1.260	88.406 ± 0.713	11.594 ± 0.713
SE.R.B	100 ± 0	0	95.351 ± 1.304	4.649 ± 1.304	94.597 ± 1.082	5.403 ± 1.082	96.841 ± 0.133	3.159 ± 0.133	90.087 ± 0.057	9.913 ± 0.057
SE.R.C	99.616 ± 0.542	0.384 ± 0.542	97.260 ± 0.174	2.740 ± 0.174	94.595 ± 0.735	5.405 ± 0.735	95.306 ± 1.008	4.694 ± 1.008	88.946 ± 2.176	11.054 ± 2.176
SE.R.90B	98.733 ± 0.199	1.267 ± 0.199	94.655 ± 0.273	5.345 ± 0.273	95.354 ± 0.978	4.646 ± 0.978	96.746 ± 1.354	3.255 ± 1.354	90.522 ± 3.373	9.478 ± 3.373
SE.R.70B	99.153 ± 1.198	0.847 ± 1.198	95.652 ± 0.283	4.348 ± 0.283	95.351 ± 0.499	4.649 ± 0.499	96.419 ± 0.575	3.581 ± 0.575	88.994 ± 0.267	11.006 ± 0.267

chemical instability, it requires precautions to use it. In fact, the most common and biologically active form of trans-RSV is rapidly isomerised to the corresponding inactive compound cis-RSV. In order to assess the copolymer matrix's ability to protect RSV from this process, an accurate quantitative evaluation (% w/w) was conducted by HPLC of the two isomers' concentrations after exposure of the MPs to different stress conditions. Specifically, aliquots (500 mg) of each system were exposed to: (1) storage in tightly closed amber containers (25 ± 2 °C/RH = 60 ± 5 %) for 24 months; (2) direct exposure to ambient light and humidity (25 ± 2 °C/RH = 60 ± 5 %) for 7 days; (3) storage in a climatic chamber (40 ± 2 °C/RH = 75 ± 5 %) for 7 days; (4) direct irradiation with UV light for 6 h. After exposure to the stress conditions, RSV was extracted (2.6.) and the samples were analysed by HPLC using the method described above (2.5.).

Table 3 shows the percentage of the two isomers at following conditions: the trans isomer constitutes the majority of the RSV present. This indicates that both preparation processes (ESE and SE) did not alter the structure of the initial molecule. Furthermore, the polymeric systems produced by both ESE and SE techniques effectively protected RSV from the various stress conditions (1,2,3,4). In fact, the maximum percentage of trans-RSV that turned into cis-RSV was 11 % at direct irradiation with UV light for 6 h. Therefore, the samples showed excellent stability.

3.5. Dissolution and release studies of RSV by polymeric MPs and kinetic analysis

In order to evaluate the dissolution rate of RSV and its release from the MPs as a function of pH, a gelatin capsule containing a known amount of drug (2.5 g) was subjected to the dissolution test (paddle method) that mimicked the physiological circumstances of the GI tract. The capsule was immersed in SGF for 2 h, simulating the stomach environment. Subsequently, the pH was adjusted to a value of 6.8 to replicate the passage from the stomach to the intestine (SIF). After a further 4 h, the pH was adjusted to 7.4, simulating the colonic environment (SCF). The dissolution profile was monitored for a total of 24 h, with 1 ml withdrawals of the various dissolution media at set intervals. The results of the dissolution profile (Fig. 3) show a high solubility of RSV, reaching 79 % already in the gastric environment. Furthermore, the solubility remains constant until a pH of 7.4 is reached, suggesting stable dissolution during passage in the colon.

The procedure described above was also performed to assess the ability of polymeric systems to withstand the physiological environment of the stomach and small intestine and to sustained drug release into the colon by studying the pH-dependent release profile of RSV. The studies were conducted on both T0 and T24 samples in order to compare the drug release profiles from both polymeric systems.

Fig. 4 shows that in the first 2 h, the system released a maximum of 20 % of the drug, demonstrating considerable resistance to the acidic environment. Subsequently, a slight gradual increase in RSV release corresponding to a maximum of 26 % was observed at pH 6.8, confirming that the system is resistant to the simulated small intestine environment. On the other hand, as can be observed after 6 h, at pH 7.4 the ESE.R.B system showed a gradual increase in RSV release from 29 % (in 30 min) to 60 % (in 10 h) reaching the plateau phase up to 24 h providing a constant release of the drug. Thus, the ESE.R.B system was optimal for ensuring a colonic release.

The corresponding SE system (Fig. 5) was shown to protect RSV from simulated gastric conditions with a release of less than 20 %. However, at pH 6.8, after 3h, drug release increased progressively to about 80 %. Subsequently, at pH 7.4, the polymer system showed greater control of the release of the active in the colonic environment reaching a plateau of over 90 % at 10h.

The near overlap of the T0 profiles with the corresponding T24 profiles (Figs. 4 and 5) shows a high stability of the polymeric systems during long-term storage under ambient conditions.

Fig. 6 shows that the release of RSV by ESE.R.C was lowest at acidic

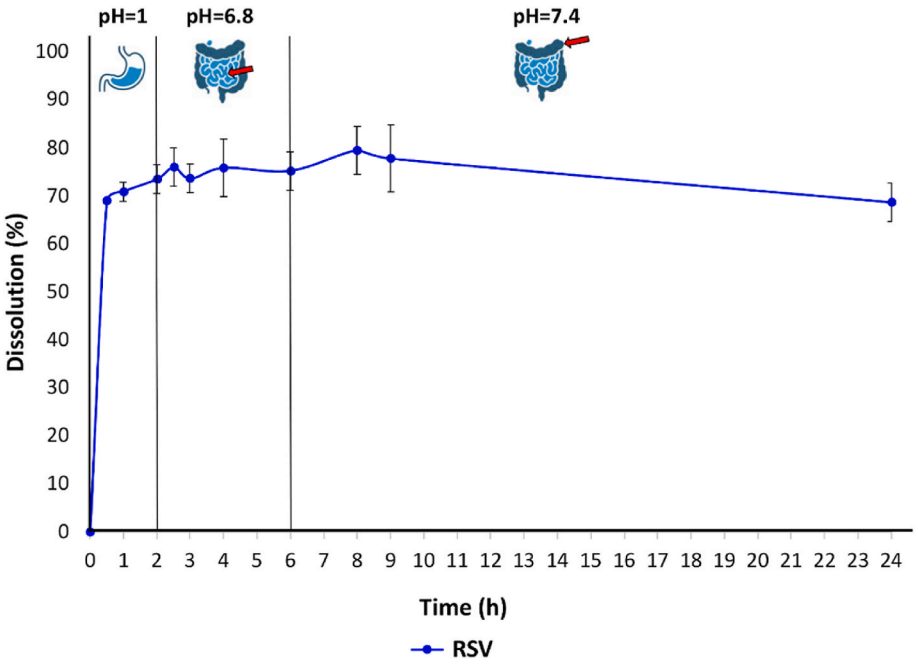

Fig. 3. The dissolution profile of RSV in in SGF (pH 1), in SIF (pH 6.8) and in SCF (pH 7.4).

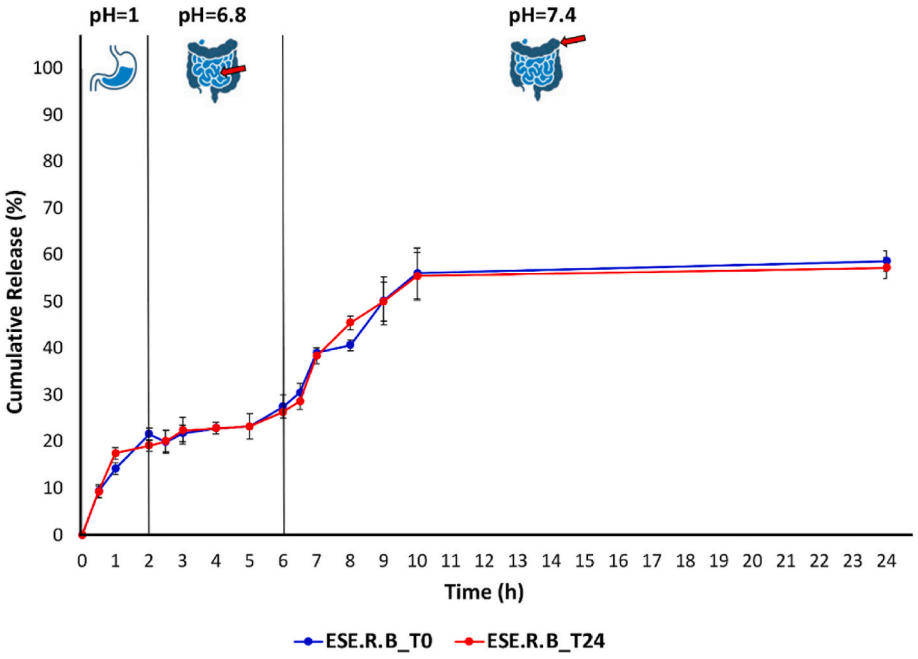

Fig. 4. The release profile of RSV from ESE.R.B_T0 and in ESE.R.B_T24 in SGF (pH 1), in SIF (pH 6.8) and in SCF (pH 7.4).

pH and increased at pH 6.8, remaining constant even at pH 7.4, ensuring a constant and prolonged release of 25 % over 24 h. The amount of RSV released and the presence of a 24-h background body suggest an extremely compact nature of the MPs.

The SE.R.C. system (Fig. 7) protected the active from the simulated gastric conditions, releasing less than 20 % in this environment. However, it showed a greater release of RSV at pH 6.8, because it is more sensible at this pH [29], which stabilised at pH 7.4 with a plateau of 80 % after 6 h and remained so over 24 h. This was probably due to the presence of Aerosil® F200, which allowed the formation of more porous particles. The greater solvent flow at the inlet ensures more effective dissolution of the drug, resulting in increased RSV release. Again, the near overlap of the T0 profiles with the corresponding T24 profiles (Figs. 6 and 7) allows us to state the high stability of the systems produced.

With the aim of optimising the release of RSV from MPs, polymeric systems were developed to ensure a prolonged release of drug into the colon and low release in SGF and in SIF. Thus, MPs containing EUG-B and EUG-C were created with weight ratios of 90:10 (ESE.R.90B) and 70:30 (ESE.R.70B). The RSV release profiles of these polymer systems showed that even a modest amount (10 % w/w) of EUG-C polymer appeared to influence the release of RSV from the system (Fig. 8). The ESE.R.90B system showed that gastric drug release always remained below 20 %, as in the previous single-polymer systems. On the other

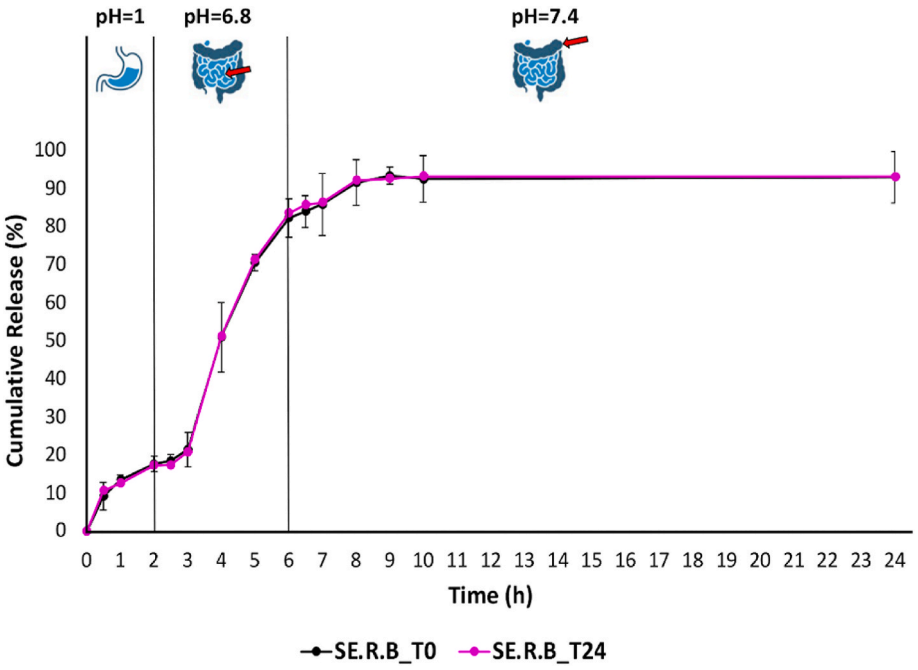

Fig. 5. The release profile of RSV from SE.R.B_T0 and in SE.R.B_T24 in SGF (pH 1), in SIF (pH 6.8) and in SCF (pH 7.4).

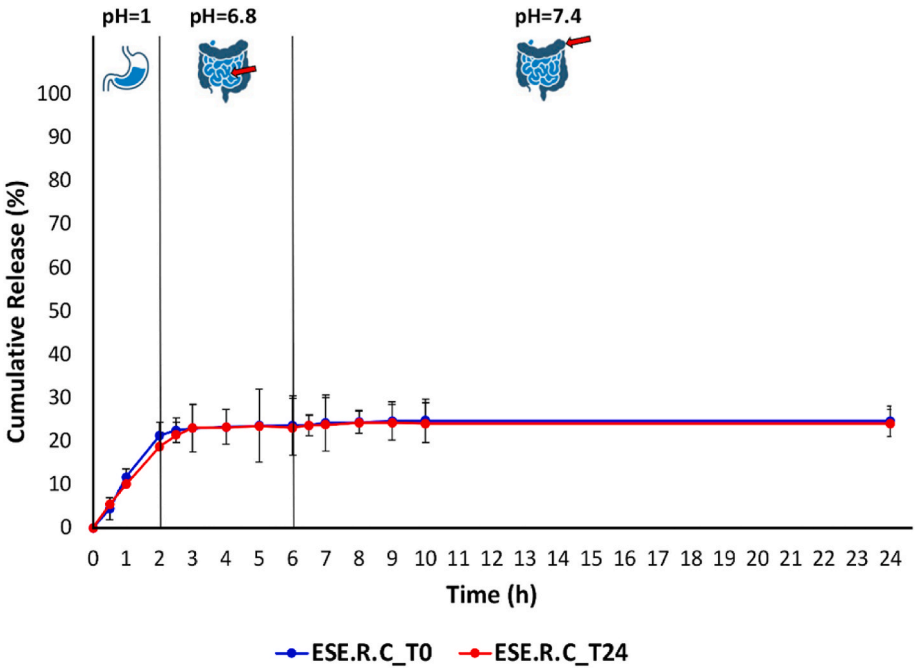

Fig. 6. The release profile of RSV from ESE.R.C_T0 and in ESE.R.C_T24 in SGF (pH 1), in SIF (pH 6.8) and in SCF (pH 7.4).

hand, compared to the EUG-B-only systems, it showed a lower and controlled release at pH 6.8 (30 %), avoiding too high releases of drug in the simulated small intestine, and an increase in release (almost 50 %) at pH 7.4 reaching the plateau phase at 7h ensuring a constant release of RSV over 24h. In addition, the ESE.R.90B system compared to the system consisting only of EUG-C showed increased drug release into the colon (target site).

The MPs obtained with the SE technique showed a more favourable release profile for the purposes of our study (Fig. 9), as SE.R.90B exhibited lower RSV release in SGF and SIF (non-target sites) than the other systems. Indeed, in SGF, the amount of RSV detected was around 10 %, while at pH 6.8 it reached 20 %. This ensures that RSV is not dispersed to non-target sites and the amount of RSV loaded will then be released at the target site (colon). Indeed, the MPs, in the simulated colon environment, showed an increase in RSV release already after 30 min reaching 40 % after 7 h and progressively increasing to a maximum peak (80 %) after 9 h (plateau phase). Thus, a considerable release corresponding to 80 % RSV remained constant for 24h. This ensures that a good dose of the active ingredient is released at the target site over a prolonged period. The release test conducted on SE.R.90B_T24 confirmed the stability of the systems as the release profiles were superimposable. This showed that the prepared polymeric systems were able to maintain their properties over time and protected the RSV from the external environment, even under prolonged storage conditions.

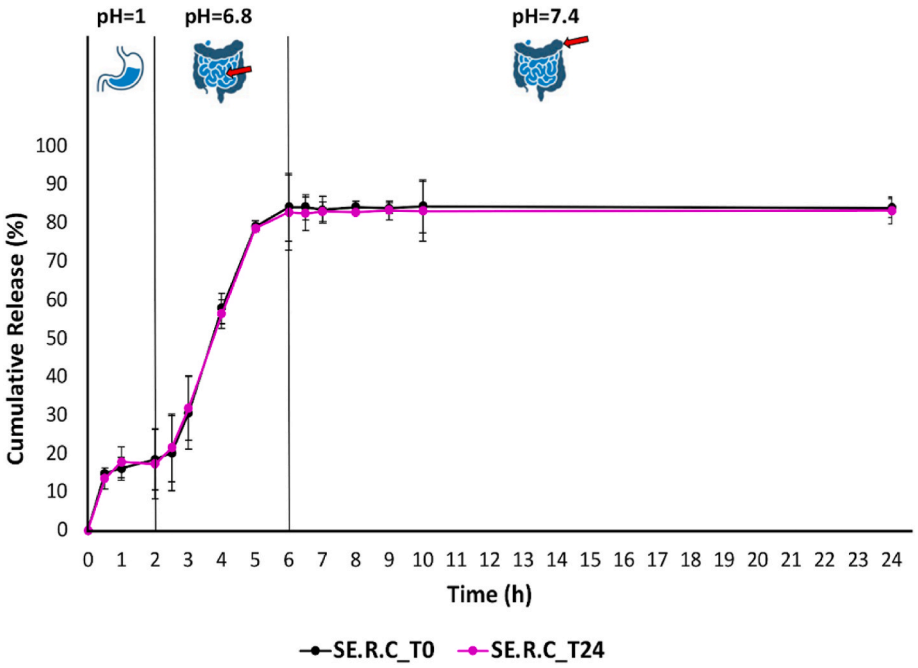

Fig. 7. The release profile of RSV from SE.R.C_T0 and in SE.R.C_T24 in SGF (pH 1), in SIF (pH 6.8) and in SCF (pH 7.4).

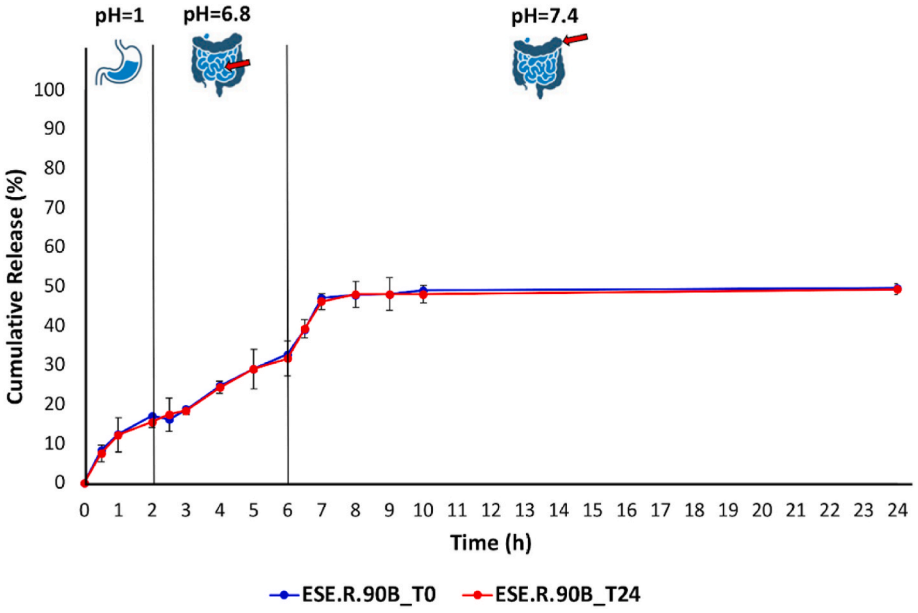

Fig. 8. The release profile of RSV from ESE.R.90B_T0 and in ESE.R.90B_T24 in SGF (pH 1), in SIF (pH 6.8) and in SCF (pH 7.4).

This characteristic is of paramount importance to ensure the effectiveness and safety of the systems themselves.

The ESE.R.70B system had an even higher percentage of the polymer EUG-C compared to the previous one. The RSV release profile from this system (Fig. 10) showed remarkable similarities with that of ESE.R.90B. However, unlike the latter, less pronounced fluctuations in release of the active ingredient were observed between the small intestine and the colon, probably due to a higher percentage of EUG-C.

The analysis conducted on the corresponding systems produced using the SE technique (Fig. 11) showed a low release (15 %) in an acidic environment and a more rapid release, reaching 50 %, at pH 6.8 as there were larger quantities of EUG-C, which is sensitive to this pH [29]. The release continued to increase (70 %) at pH 7.4 reaching the plateau phase in 7h.

Comparison of the release profiles of the T0 MPs with those of T24 provided further confirmation of the high stability of the systems produced.

In brief, EUGC and EUGB are derivatives of pH-sensitive methacrylate copolymers. These polymers contain ionisable groups that, depending on the pH of the environment, undergo different degrees of ionisation, thus influencing the solubility and conformation of the polymer. In particular, when the monomers of polymer chains become electrically charged at a certain pH value, the interaction with water molecules increases. Consequently, the solubility of the polymer is directly proportional to the degree of interaction between the polymer chains and water: depending on this, the polymer may be partially or completely soluble in a medium with a certain pH [41].

As already extensively mentioned, EUGC is most sensitive at pH

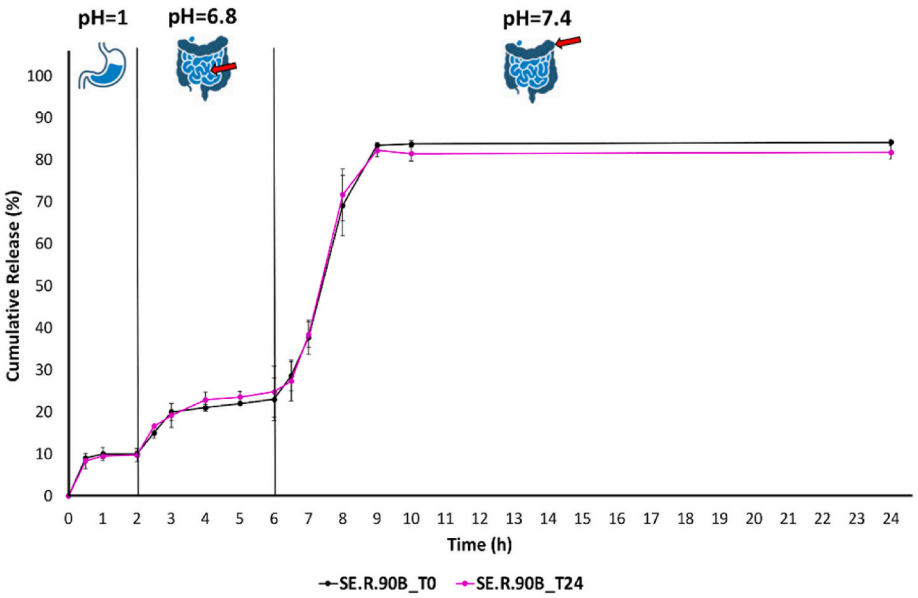

Fig. 9. The release profile of RSV from SE.R.90B_T0 and in SE.R.90B_T24 in SGF (pH 1), in SIF (pH 6.8) and in SCF (pH 7.4).

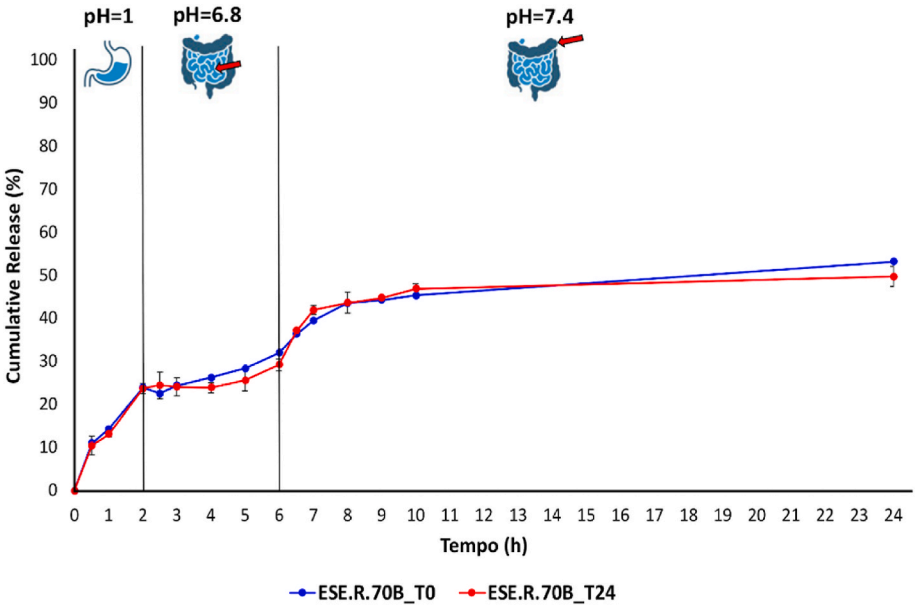

Fig. 10. The release profile of RSV from ESE.R.90B_T0 and in ESE.R.90B_T24 in SGF (pH 1), in SIF (pH 6.8) and in SCF (pH 7.4).

values $\geq$ 6.8, whereas EUGB is most sensitive at pH values $\geq$ 7.4. Combining the two polymers in a 90:10 ratio (SE.R.90B) resulted MPs with reduced RSV release at pH 6.8, due to the greater presence of EUGB, which is less sensitive at this pH. At the same time, the presence of EUGC contributes to a more porous structure of the MPs and a higher drug release in the colon, due to its higher sensitivity at pH $\geq$ 6.8, which leads to a gradual dissolution or erosion of the polymer matrix.

The release rate constants of the polymeric systems prepared by ESE and SE techniques were estimated from the slope of the different curves and the regression values (R^2) obtained. In vitro release of RSV from MPs was performed in different artificial fluids and the kinetic profile was evaluated in SIF (3 h–6 h) and SCF (7 h–24 h). Table 4 shows the regression coefficient (R^2) values of the polymeric systems prepared with the ESE technique and Table 5 shows the R^2 of those prepared with the SE technique. The following samples ESE.R._T0, ESE.R.B_T24, ESE.R.70B_T0, ESE.R.70B_T24, SE.R.70B_T0 and SE.R.70B_T24 in SIF showed that the R^2 that best described the kinetic profile of RSV was the first-order equation, suggesting that drug release was concentration-dependent [42]. Furthermore, the drug released from samples SE.R.90B_T0 and ESE.R.C_T0 in SIF was best described by the zero-order equation, indicating that drug release was independent of its concentration [42]. Furthermore, the best R^2 value obtained for RSV released from ESE.R.C_T0, ESE.R.90B_T0, ESE.R.90B_T24, SE.R.B_T0, SE.R.B_T24, SE.R.C_T0, SE.R.C_T24 and SE.R.90B_T24 in SIF was described by Hixson-Crowell, indicating that the release of RSV is due to a dissolution process with a change in the diameter and surface area of the scaffold [31,43]. The release of RSV from ESE.R.C_T24 in SIF was better described by the Higuchi equation, showing that the diffusion of the drug slows down with increasing diffusion distance [32,44]. Finally, the RSV released from all samples (ESE.R.B_T0, ESE.R.B_T24, ESE.R.C_T0, ESE.R.C_T24, ESE.R.90B_T0, ESE.R.90B_T24, ESE.R.70B_T0, ESE.R.70B_T24, SE.R.B_T0, SE.R.B_T24, SE.R.C_T0, SE.R.C_T24, SE.R.90B_T0, SE.

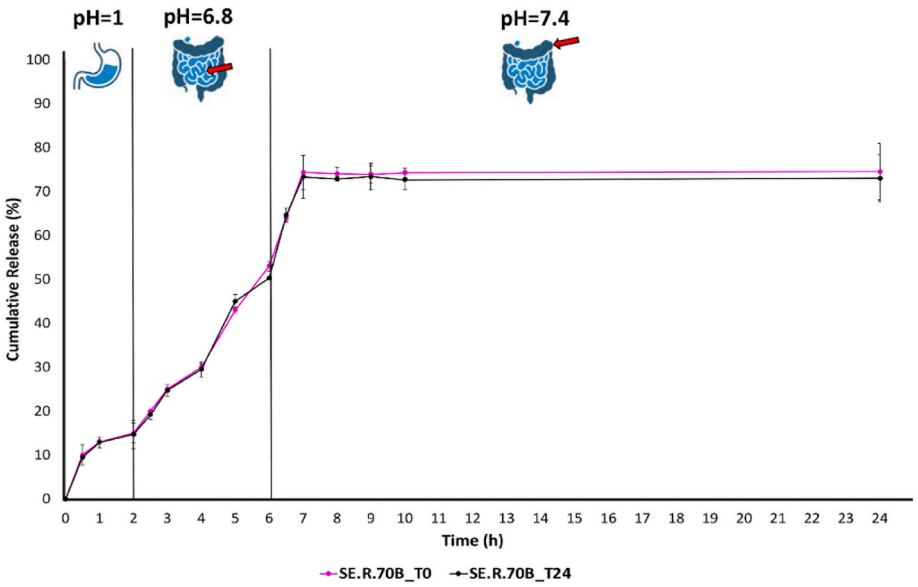

Fig. 11. The release profile of RSV from SE.R.70B_T0 and in SE.R.70B_T24 in SGF (pH 1), in SIF (pH 6.8) and in SCF (pH 7.4).

Table 4
Regression coefficient values (R^2) for different release kinetic models obtained from release kinetic profile of ESE.R.B_T0, ESE.R.B_T24, ESE.R.C_T0, ESE.R.C_T24, ESE.R.90B_T0, ESE.R.90B_T0, ESE.R.70B_T24, ESE.R.70B_T24 in SIF and in SCF. The calculations were made on the linear part of the release curves (from 3 h to 6 h in SIF and from 7 h to 24 h in SCF forward).

SIF (pH 6.8)					
Samples (ESE)	Zero order	First order	Higuchi	Hixson-Crowell	Korsmeyer-Peppas
ESE.R.B_T0	0.8097	0.8274	0.3746	0.8059	0.3782
ESE.R.B_T24	0.7806	0.7924	0.3277	0.7782	0.3274
ESE.R.C_T0	0.9172	0.9168	0.0749	0.9172	0.0760
ESE.R.C_T24	0.0909	0.0911	0.2284	0.0909	0.2277
ESE. R.90B_T0	0.9873	0.9629	0.2134	0.9912	0.2232
ESE. R.90B_T24	0.9716	0.9453	0.1748	0.9763	0.1897
ESE. R.70B_T0	0.9733	0.9846	0.2305	0.9698	0.2272
ESE. R.70B_T24	0.7962	0.8048	0.1579	0.7940	0.1455
SCF (pH 7.4)					
Samples (ESE)	Zero order	First order	Higuchi	Hixson-Crowell	Korsmeyer-Peppas
ESE.R.B_T0	0.5287	0.4986	0.5781	0.5499	0.6069
ESE.R.B_T24	0.4884	0.4533	0.5415	0.5104	0.5701
ESE.R.C_T0	0.2490	0.2494	0.2952	0.2490	0.3534
ESE.R.C_T24	0.0005	0.0006	0.0030	0.0004	0.0098
ESE. R.90B_T0	0.6513	0.6458	0.6989	0.6547	0.7479
ESE. R.90B_T24	0.5997	0.5880	0.6389	0.6068	0.6732
ESE. R.70B_T0	0.9033	0.8709	0.9289	0.9191	0.9306
ESE. R.70B_T24	0.7841	0.7646	0.8252	0.7948	0.8538

R.90B_T24, SE.R.70B_T0 and SE.R.70B_T24) in SCF was best described by the Korsmeyer-Peppas equation. The n-parameters (diffusional release exponent) of all polymeric systems in SCF (Table 6) are $n < 0.45$, indicating a quasi-Fickian diffusion profile of RSV release. This is predominantly a diffusional phenomenon with a slight case of polymer swelling [31,44,45].

4. Conclusions

The aim of this project was to develop food grade Eudraguard® Biotic/Control MPs loaded with RSV, comparing two green techniques, ESE and SE, to preserve the trans-RSV isomer (the isoform with greater anti-inflammatory activity than cis) and ensure sustained release in the colon for the treatment of IBD. Both techniques proved to be effective for MP development. In fact, HPLC analyses conducted on T0 samples prepared with the ESE and SE techniques showed that the amount of encapsulated RSV was mainly in the trans form, indicating that the formulation conditions do not induce isomerization.

Furthermore, the polymeric systems prepared by ESE and SE techniques showed good encapsulation of RSV (%EE > 62). These data were further confirmed by FT-IR analysis of the T0 and T24 systems, which showed that the RSV was homogeneously dispersed in the polymer matrix, the polymer functional groups did not change and the active ingredient was effectively encapsulated. Furthermore, they showed high system stability during the 24-month storage period. These data were again confirmed by the low percentage of cis-RSV detected (%cis-RSV <11), by HPLC analysis, after exposure of the samples to different stress conditions, demonstrating the ability of the polymer matrix to prevent the RSV isomerization process.

Finally, in vitro studies demonstrated the ability of the polymeric matrices to protect RSV from the gastric environment, avoiding a high release in the stomach and intestine and ensuring a higher and prolonged release (up to 24 h) in the colonic tract (target site). In particular, SE.R.90B_T0 and SE.R.90B_T24 showed a preferable RSV release profile for our purpose, as RSV release did not exceed 10 % in the stomach and 20 % in the small intestine (non-target sites). Instead, the maximum peak (80 %) in the simulated colonic environment (target site) was reached progressively after 9 h (plateau phase) and remained constant until 24 h.

Further in vitro and in vivo studies will be conducted to investigate the safety and therapeutic effects of these systems.

CRediT authorship contribution statement

Rosamaria Lombardo: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Writing – review & editing. **Salvatore Rizzo:** Investigation, Formal analysis, Data curation. **Jarkko Rautio:** Writing – review & editing, Validation, Supervision, Formal analysis. **Rosario Pignatello:** Writing –

Table 5
Regression coefficient values (R^2) for different release kinetic models obtained from release kinetic profile of SE.R.B_T0, SE.R.B_T24, SE.R.C_T0, SE.R.C_T24, SE.R.90B_T0, SE.R.90B_T0, SE.R.70B_T24, SE.R.70B_T24 in SIF and in SCF. The calculations were made on the linear part of the release curves (from 3 h to 6 h in SIF and from 7 h to 24 h in SCF forward).

SIF (pH 6.8)					
Samples (SE)	Zero order	First order	Higuchi	Hixson-Crowell	Korsmeyer-Peppas
SE.R.B_T0	0.9629	0.8775	0.2005	0.9968	0.2396
SE.R.B_T24	0.9635	0.8750	0.2009	0.9978	0.2412
SE.R.C_T0	0.9279	0.8713	0.1457	0.9654	0.1892
SE.R.C_T24	0.9296	0.8834	0.1250	0.9577	0.1675
SE.R.90B_T0	1	0.9995	0.2	0.9999	0.2002
SE.R.90B_T24	0.8758	0.8556	0.3491	0.8796	0.3480
SE.R.70B_T0	0.9745	0.9839	0.1165	0.9675	0.1102
SE.R.70B_T24	0.9509	0.9517	0.0568	0.9511	0.0605
SCF (pH 7.4)					
Samples (SE)	Zero order	First order	Higuchi	Hixson-Crowell	Korsmeyer-Peppas
SE.R.B_T0	0.1929	0.1912	0.2303	0.2033	0.2767
SE.R.B_T24	0.1893	0.1874	0.2275	0.2023	0.2746
SE.R.C_T0	0.0033	0.0034	0.0103	0.0029	0.0243
SE.R.C_T24	0.2440	0.2440	0.2651	0.2440	0.2891
SE.R.90B_T0	0.2313	0.2081	0.2747	0.2619	0.3016
SE.R.90B_T24	0.1979	0.1823	0.2377	0.2181	0.2694
SE.R.70B_T0	0.4021	0.4012	0.3765	0.4038	0.3418
SE.R.70B_T24	0.0447	0.0443	0.0566	0.0453	0.0716

Table 6
n (diffusional release exponent) parameters of ESE.R.B_T0, ESE.R.B_T24, ESE.R.C_T0, ESE.R.C_T24, ESE.R.90B_T0, ESE.R.90B_T24, ESE.R.70B_T0, ESE.R.70B_T24, SE.R.B_T0, SE.R.B_T24, SE.R.C_T0, SE.R.C_T24, SE.R.90B_T0, SE.R.90B_T24, SE.R.70B_T0 and SE.R.70B_T24 samples in SCF.

Samples in SCF	n (diffusional release exponent)
ESE.R.B_T0	0.295
ESE.R.B_T24	0.251
ESE.R.C_T0	0.010
ESE.R.C_T24	0.001
ESE.R.90B_T0	0.036
ESE.R.90B_T24	0.039
ESE.R.70B_T0	0.213
ESE.R.70B_T24	0.124
SE.R.B_T0	0.037
SE.R.B_T24	0.034
SE.R.C_T0	0.001
SE.R.C_T24	0.003
SE.R.90B_T0	0.389
SE.R.90B_T24	0.351
SE.R.70B_T0	0.004
SE.R.70B_T24	0.002

review & editing, Supervision, Resources, Project administration, Conceptualization.

Funding sources

Rosamaria Lombardo was supported by the PhD program in Neurosciences, XXXVI cycle, University of Catania (Italy).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are grateful to NANOMED (the Research Centre for Nanomedicine and Pharmaceutical Nanotechnology of the University of Catania) and to UEF analytical laboratory (Kuopio, Finland) for technical assistance.

Data availability

Data will be made available on request.

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