

## Article

# Mucoadhesive Buccal Patches Containing Resveratrol and/or Erythromycin-Loaded Lipid Microparticles as a Potential Targeted Strategy for the Prevention and Management of MRONJ in Patients Undergoing Oral Surgery

Giulia Di Prima <sup>1</sup>, Cecilia La Mantia <sup>2</sup>, Giada Tranchida <sup>3</sup>, Alessandro Presentato <sup>1</sup>, Giovanna Giuliana <sup>2</sup>,  
Giuseppina Campisi <sup>4</sup> and Viviana De Caro <sup>1,\*</sup>

<sup>1</sup> Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Via Archirafi 32, 90123 Palermo, Italy; giulia.diprima@unipa.it (G.D.P.); alessandro.presentato@unipa.it (A.P.)

<sup>2</sup> Department of Precision Medicine in Medical, Surgical and Critical Care (Me.Pre.C.C.), University of Palermo, Via Liborio Giuffrè 5, 90127 Palermo, Italy; cecilia.lamantia@unipa.it (C.L.M.); giovanna.giuliana@unipa.it (G.G.)

<sup>3</sup> Department of Engineering, University of Palermo, Viale delle Scienze, 90128 Palermo, Italy; giada.tranchida@unipa.it

<sup>4</sup> Department of Biomedicine, Neurosciences and Advanced Diagnostics (BIND), University of Palermo, Via del Vespro 129, 90127 Palermo, Italy; giuseppina.campisi@unipa.it

\* Correspondence: viviana.decaro@unipa.it; Tel.: +39-091-23891926

## Abstract

**Background/Objectives:** Oral surgical procedures in patients at risk of/ diagnosed with MRONJ require systemic antibiotic therapy, which can fail to achieve an adequate local drug concentration. This research aims to design mucoadhesive buccal patches (containing erythromycin or the erythromycin–resveratrol combination) tailored to the therapeutic needs of patients at risk of MRONJ undergoing oral surgery. **Methods:** Erythromycin (ERY) and resveratrol (RSV) were embedded into lipid-based microparticles prepared via hot melt dispersion. The microparticles, recovered in the form of dry powders, were characterized in terms of yield, softening/melting temperature, active(s) content, physical state (amorphous vs. crystalline), and individual and bulk properties. Then, they were loaded into a hydrophilic gel, which was dried, obtaining microparticle-loaded buccal patches. The optimized patches were characterized in terms of uniformity, folding endurance, swelling, mucoadhesion, and oromucosal permeation/retention. **Results:** The microparticles were efficiently produced via a green approach, resulting in reproducible pharmaceutical powders with high loading efficacy ( $\approx 90\%$ ), spherical morphology, particle sizes in the range of approximately 106–425  $\mu\text{m}$ , and a softening temperature close to body temperature. The buccal patches were also obtained via a green approach, and were found to be thin, flexible, homogeneous, highly swellable, extremely mucoadhesive, and able to promote ERY and RSV accumulation in the buccal tissue ( $\approx 25\%$  and  $2\%$  of ERY and RSV, respectively, after 2 h) while avoiding active(s) absorption. **Conclusions:** The proposed buccal patches are viable candidates for further clinical trials aimed at evaluating both the effectiveness of locoregional antibiotic treatment and the usefulness of the co-administration of RSV and ERY.

**Keywords:** buccal delivery; oromucosal formulation; MRONJ; loco-regional delivery; microcomposite patches; solid lipid microparticles; microstructured lipid carrier; mucoadhesion



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## 1. Introduction

Medication-Related Osteonecrosis of the Jaw (MRONJ) is a severe bone disorder, first described in 2003, that affects the maxillary and mandibular bones. Its etiology is multifactorial, involving both structural and anatomical aspects of the oro-maxillo-facial region, as well as alterations of the immune and bone microenvironment [1]. The primary pharmacological agents associated with MRONJ development include: (i) bisphosphonates, e.g., zoledronate, which bind to hydroxyapatite in the bone extracellular matrix, promoting the adhesion of bacterial biofilm and predisposing the jawbone to MRONJ development [2]; (ii) inhibitors of the receptor activator of nuclear factor  $\kappa$ B ligand (RANKL), e.g., denosumab [3]; and (iii) anti-angiogenic agents [4]. Epidemiological data indicate that the risk of developing MRONJ is markedly higher in cancer patients, who are often exposed to multiple risk factors simultaneously [5]. In this context, invasive dental procedures (e.g., tooth extractions and implant placement), as well as dental/periodontal infective diseases (leading to the tooth extraction), are recognized as events triggering MRONJ onset [6]. These procedures directly expose the jawbone to bacterial colonization and compromise the integrity of the oral mucosa, which normally acts as a protective barrier, thereby promoting the development of bone necrosis [7].

According to the protocol promoted by the Italian Society of Oral and Maxillofacial Surgery (SICMF) and the Italian Society of Oral Pathology and Medicine (SIPMO), surgical procedures (e.g., dental extractions, periodontal or endodontic surgery) in patients at risk of or diagnosed with MRONJ require combined medical prophylaxis and standardized surgical techniques. The recommended regimen includes a 0.12% chlorhexidine mouthwash, together with systemic antibiotic therapy initiated one day before surgery and continued for at least six days postoperatively [8,9].

Although the precise role of infection in MRONJ pathogenesis remains incompletely understood, microbial colonization is consistently recognized as a constant clinical feature, thereby justifying the routine use of antibiotics to control local signs and symptoms. The most established regimens combine penicillin and metronidazole; alternatively, erythromycin, clindamycin, or ciprofloxacin may be used in patients with a  $\beta$ -lactams allergy or in cases of contraindications, such as renal impairment or drug tolerance [10,11]. Despite the benefits of systemic antibiotic therapy, achieving adequate local drug concentrations is often challenging due to reduced vascularization of necrotic bone tissue, which limits antibiotic delivery. Consequently, topical antimicrobial administration directly at the surgical site is considered advantageous, allowing high loco-regional concentrations while minimizing or eliminating systemic side effects. Furthermore, recent literature suggests that natural bioactive molecules may serve as adjuvants, enhancing the therapeutic efficacy of the drug. In this regard, resveratrol (RSV)—a polyphenolic phytoalexin with osteoinductive, antibiofilm, antioxidant, and wound-healing properties—has attracted increasing attention [12–14]. In recent decades, research has increasingly focused on advanced oral drug delivery systems, such as mucoadhesive patches and films. These systems are designed to adhere to the oral mucosa, thereby improving therapeutic efficacy, particularly for loco-regional treatments. These thin, flexible formulations are capable of adhering to the oral mucosa and releasing the drug in a controlled manner, offering several advantages, such as prolonged contact time, increased local bioavailability, drug protection, ease of application, patient comfort, and precise dosing [15]. Moreover, such systems act both as protective barriers and drug carriers, playing a pivotal role in improving patient symptoms by protecting lesions from mechanical stress caused by tongue movements [16].

In light of these considerations, this research aimed to design and optimize novel buccal drug delivery systems (BDDSs) in the form of mucoadhesive patches, specifically tailored to the therapeutic needs of patients at risk of MRONJ undergoing oral surgery,

exploiting the oromucosal route as a potentially targeted, patient-friendly, and non-invasive strategy. Considering the physicochemical properties of the active compounds, the therapeutic agents were first encapsulated in lipid microparticles, which were subsequently entrapped in a hydrophilic polymeric matrix to obtain the desired final formulation. Moreover, in view of a future clinical evaluation aimed at assessing the potential synergistic effect of antibiotic and resveratrol co-administration, two types of patches were developed and characterized: one loaded with erythromycin alone (ERY) and another containing the ERY + RSV combination. The choice of ERY as the antibiotic was guided by clinical, formulation, and regulatory considerations, including the future possibility of enrolling penicillin-sensitive patients, who represent a relevant clinical subgroup.

## 2. Results and Discussion

### 2.1. Lipid-Based Microparticles Preparation and Characterization

One of the main challenges in the delivery of lipophilic molecules is their poor solubility in aqueous media, which severely compromises absorption, bioavailability, and, ultimately, therapeutic efficacy. This limitation is evident in the case of erythromycin (ERY) and resveratrol (RSV). This issue imposes significant limitations on oromucosal drug delivery, as the oral cavity is characterized by a highly aqueous salivary environment and polar mucosal tissues. These conditions markedly hinder the interaction between the tissue and lipophilic compounds unless the latter are molecularly dispersed. To address these limitations, lipid-based delivery systems were investigated in the present study. To enable ERY delivery, solid lipid microparticles (SLM-ERY) were developed based on our previously patented SLM composition and preparation method (n. IT 201900011436), with modifications aimed at achieving a high ERY loading capacity. For RSV delivery, microstructured lipid carriers (MLC-RSV) were designed using the same formulation platform, developing a mixed solid–liquid lipid matrix. In this case, Labrasol® (PEG-8 Caprylic/Capric Glycerides) was included in the lipid phase to promote RSV solubilization within the microparticles [17].

In designing microparticles for oromucosal application, their softening and melting temperatures are critical. Excessively low softening temperatures hinder handling, storage, and transport, whereas excessively high values prevent drug release at the target site via fusion and partitioning into the tissues. To achieve the intended outcome, the lipid components ratio must be carefully evaluated. Specifically, for the SLM-ERY, the 1-hexadecanol–cetyl decanoate ratios were chosen according to previous results [18], while for the MLC-RSV, several compositions containing Labrasol® were tested. Microparticles were produced using the hot-melt dispersion technique. This solvent-free method, involving melting of the lipid phase, dispersion into a preheated aqueous phase under high-shear mixing, and subsequent rapid cooling, proved highly effective in ensuring reproducible particle formation and efficient drug encapsulation. Beyond its operational simplicity and industrial scalability, the process also demonstrated an environmentally sustainable profile, as the absence of organic solvents eliminated the risk of toxic residues in the final formulation. To implement the described method, the following parameters were optimized: (i) the lipid-to-aqueous phase ratio; (ii) the speed of agitation; (iii) the time of hot and cold agitation; and (iv) the composition of the external aqueous phase. The latter consisted of a saturated NaCl solution for both SLM-ERY and MLC-RSV preparations in order to minimize the solubility of ERY and RSV in the external phase through the salting-out effect and thus maximize drug loading. This approach was particularly beneficial for MLC-RSV as the presence of Labrasol® in the lipid matrix could otherwise enhance RSV aqueous solubility due to its surfactant properties [19]. Table 1 reports the results obtained in terms of the yield of the preparation process, as well as softening and melting temperatures.

**Table 1.** Yield % ( $n = 5$ ) and softening and melting points ( $n = 10$ ) of SLM-ERY and MLC-RSV. Means  $\pm$  SE.

| <i>SLM-ERY</i> |                |                    |                  |
|----------------|----------------|--------------------|------------------|
| Formula Code   | Yield %        | Softening point °C | Melting point °C |
| SLM-ERY-10     | 97.8 $\pm$ 0.3 | 36.1 $\pm$ 0.2     | 46.0 $\pm$ 0.1   |
| SLM-ERY-15     | 97.6 $\pm$ 0.3 | 33.7 $\pm$ 0.3     | 45.1 $\pm$ 0.4   |
| SLM-ERY-25     | 97.3 $\pm$ 0.5 | 32.5 $\pm$ 0.1     | 43.9 $\pm$ 0.1   |
| <i>MLC-RSV</i> |                |                    |                  |
| Formula Code   | Yield %        | Softening point °C | Melting point °C |
| MLC-RSV-A      | 72.8 $\pm$ 1.2 | 43.4 $\pm$ 1.1     | 49.4 $\pm$ 0.7   |
| MLC-RSV-B      | 71.1 $\pm$ 2.3 | 42.9 $\pm$ 0.5     | 47.7 $\pm$ 0.7   |
| MLC-RSV-C      | 67.8 $\pm$ 3.0 | 38.4 $\pm$ 0.9     | 47.9 $\pm$ 0.8   |
| MLC-RSV-D      | 86.5 $\pm$ 1.5 | 37.1 $\pm$ 0.2     | 43.2 $\pm$ 0.1   |
| MLC-RSV-E      | 76.3 $\pm$ 1.7 | 35.4 $\pm$ 0.4     | 42.6 $\pm$ 0.1   |
| MLC-RSV-F      | 82.9 $\pm$ 0.7 | 34.6 $\pm$ 0.2     | 42.7 $\pm$ 0.1   |

For the SLM-ERY formulation, the production yield was very high (>97%), and the softening and melting points were consistently suitable for the intended application. Notably, increasing the ERY content in the lipid matrix led to a decrease in both softening and melting temperatures, despite the high melting point of ERY ( $\approx 190$  °C), likely due to an increased degree of structural disorder within the system. For the MLC-RSV formulations, compositions A–C, which lacked cetyl decanoate as a lipid component, exhibited low yields and relatively high softening and melting points. The inclusion of cetyl decanoate likely improved the structural consistency of the microparticles, thereby increasing the yield. Furthermore, as cetyl decanoate has a lower melting point than 1-hexadecanol (32 vs. 49.3 °C), its progressive increase in formulations D–F contributed to a reduction in the softening point, ultimately achieving the desired thermal behavior while maintaining acceptable yields (>80%). Based on these results, all subsequent assessments were restricted to MLC-RSV-F. In contrast, all SLM-ERY formulations were retained for analysis to assess the impact of increased drug loading on the other microparticle properties.

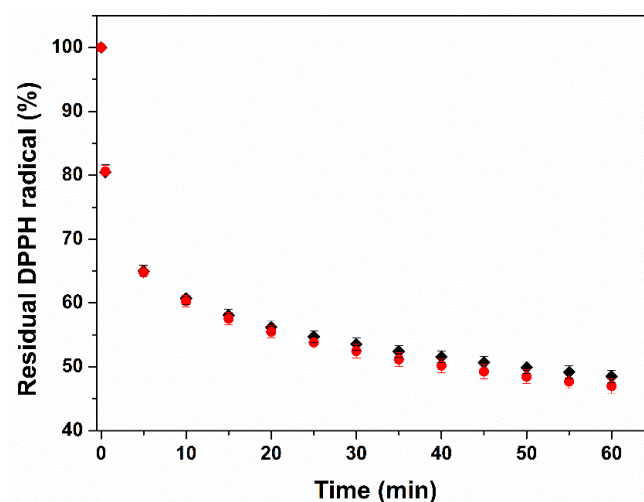
Firstly, the drug loading and loading efficacy were determined, as summarized in Table 2. The observed DL values were close to the theoretical values, resulting in high LE (>85%), thereby confirming the effectiveness of the preparation method.

**Table 2.** DL% and LE% values for the considered SLM-ERY and MLC-RSV. Means ( $n = 15$ )  $\pm$  SE.

| Formula Code | DL%              | LE%              |
|--------------|------------------|------------------|
| SLM-ERY-10   | 4.93 $\pm$ 0.04  | 98.56 $\pm$ 0.01 |
| SLM-ERY-15   | 9.87 $\pm$ 0.96  | 98.73 $\pm$ 9.60 |
| SLM-ERY-25   | 13.33 $\pm$ 0.02 | 88.85 $\pm$ 0.15 |
| MLC-RSV-F    | 22.36 $\pm$ 0.24 | 89.42 $\pm$ 0.95 |

In addition, MLC-RSV-F was subjected to a DPPH assay to assess the retention of RSV's antioxidant properties within the lipid microcarriers. According to the literature, the pharmacological potential of RSV is mainly attributed to its scavenging activity against reactive oxygen species (ROS), which results in the attenuation of the inflammatory phase and the promotion of tissue regeneration [20,21]. Given that the MLC-RSV preparation process involved exposure to high temperatures (100 °C), it was necessary to evaluate whether this affected RSV's antioxidant activity. Accordingly, the assessment was performed by comparing the DPPH scavenging activity of MLC-RSV to that of a methanolic RSV solution at a concentration matching the actual RSV content in the microparticles. Figure 1 reports the kinetics of DPPH radical consumption as a function of time up to

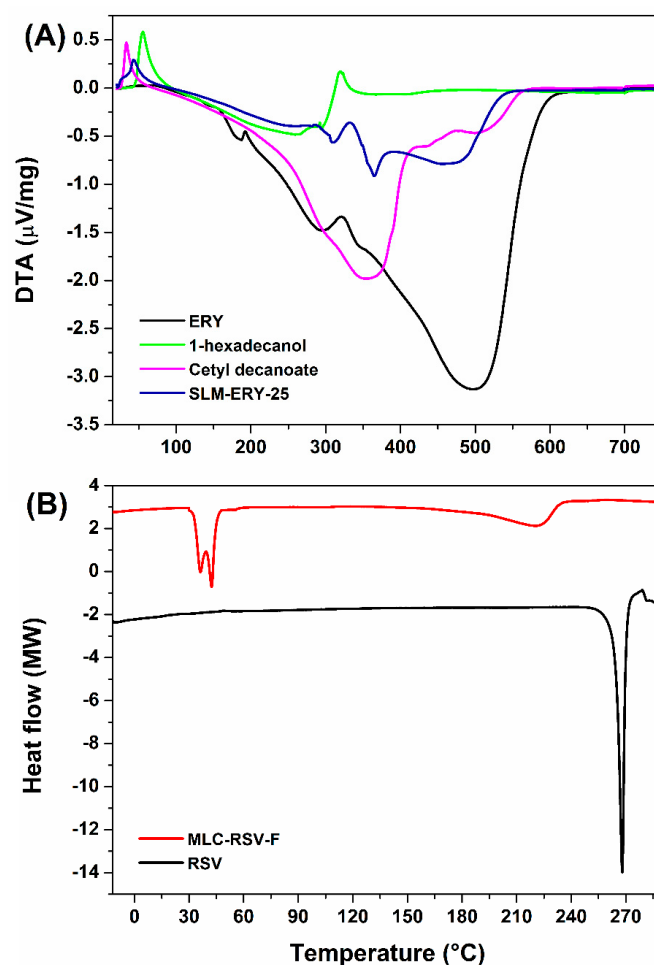
1 h. As can be observed, the sample and control curves largely overlapped, and the minor difference displayed falls within the experimental error. These findings demonstrated that RSV retained its antioxidant capacity following microencapsulation, providing additional evidence of the reliability and efficiency of the preparation process.



**Figure 1.** Residual DPPH radical (%) as a function of incubation time for MLC-RSV-F (black) compared with RSV solution at an equivalent concentration (red). Means ( $n = 3$ )  $\pm$  SE.

Furthermore, as the reported softening and melting temperature points suggest an amorphous state of both the SLM-ERY and the MLC-RSV, the crystalline or amorphous state of ERY and RSV inside the microparticles was investigated by DTA and DSC analyses, respectively. Figure 2A displays the DTA curves of ERY, 1-hexadecanol, cetyl decanoate, and SLM-ERY-25. As can be observed, the pure components of the microparticles showed peaks related to their melting points: 32, 49.3, and 190 °C for cetyl decanoate, 1-hexadecanol, and ERY, respectively, followed by their degradation peaks [18]. SLM-ERY-25's behavior was significantly different, exhibiting a peak between the two previously observed for the solid lipids used, in accordance with the melting point of the microparticles. No melting peaks due to crystalline ERY were observed, confirming the complete amorphization of the active. Figure 2B demonstrates the RSV and MLC-RSV-F thermograms. The RSV curve displayed a well-defined endothermic peak at around 270 °C, corresponding to its melting point, while the RSV-loaded microparticles also exhibited two peaks consistent with the previously observed softening ( $\approx 35$  °C) and melting ( $\approx 43$  °C) temperature points. The disappearance of the endothermic melting peak of pure RSV in the MLC-RSV sample suggests RSV's complete amorphization following encapsulation into the lipid matrix of the MLCs. Consequently, when embedded into the prepared microparticles, both ERY and RSV are in their amorphous form.

To summarize, these first characterizations highlight the achievement of the first technological goal: to design microsystems potentially able to release the actives through softening upon reaching body temperature. Indeed, as they are expected to be at body temperature only when applied to the mucosa, they should soften in close contact with the tissue, which is lipophilic, and affine to the chosen excipients that can penetrate the mucosa carrying the actives (already in their molecular form). Based on these encouraging results, the microparticles were then considered as innovative pharmaceutical powders and were thus evaluated in terms of individual and bulk properties.



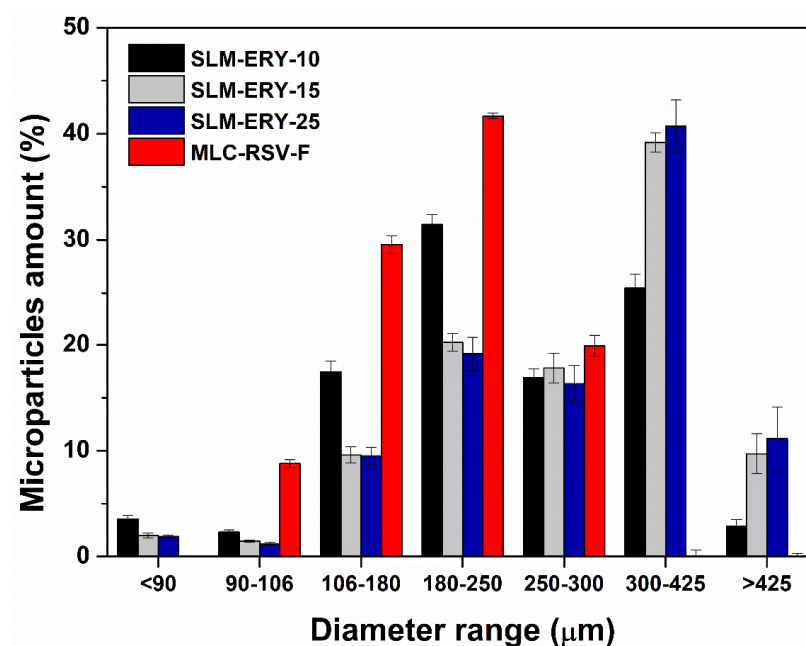
**Figure 2.** (A) DTA curves for ERY (black), 1-hexadecanol (green), cetyl decanoate (pink), and SLM-ERY-25 (blue); (B) DSC thermograms of RSV (black) and MLC-RSV-F (red).

The individual properties evaluated included particle size, particle distribution, and morphology. Particle size was determined by sieving, using standard test sieves. Figure 3 shows the percentage size distribution of SLM-ERY formulations. As can be observed, SLM-ERY-15 and SLM-ERY-25 exhibit very similar size profiles, with larger particles compared to SLM-ERY-10. For SLM-ERY-10, 90.41% of microparticles had diameters between 106 and 425  $\mu\text{m}$ , while only slightly more than 7% were below 106  $\mu\text{m}$ , and 2.22% exceeded 425  $\mu\text{m}$ . In SLM-ERY-15 and SLM-ERY-25, the fraction of particles within the range of 106–425  $\mu\text{m}$  decreased to 85.67% and 85.75%, respectively, reflecting an increased proportion ( $\sim 10\%$ ) of particles larger than 425  $\mu\text{m}$ . In both formulations, approximately 3% of the particles were below 106  $\mu\text{m}$ . Notably, the particle size distributions of all three formulations did not follow a typical Gaussian pattern but instead displayed a multimodal trend.

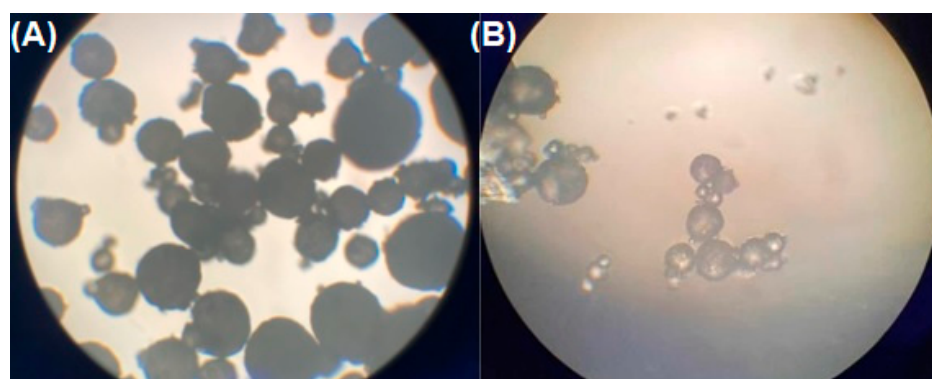
Conversely, the particle size analysis of MLC-RSV (Figure 3) revealed that 99.93% of particles had diameters between 106 and 300  $\mu\text{m}$ , exhibiting a near-Gaussian distribution. For all the formulations, powder fraction exceeding 425  $\mu\text{m}$  was discarded due to excessive size.

Morphological analysis was performed using optical microscopy. As illustrated in Figure 3, both types of particles appeared as regular spheres of varying sizes, consistent with the particle size measurements. The observed sphericity is considered favorable, as it is expected to enhance the flowability of the powders. The primary morphological distinction between the carriers lies in their appearance: SLM-ERY particles were solid and

opaque (Figure 4A), whereas MLC-RSV particles appeared translucent (Figure 4B), likely reflecting their mixed solid–liquid lipid composition.



**Figure 3.** Particle size distribution (%) of SLM-ERY and MLC-RSV, determined by the sieving method. Means ( $n = 5$ )  $\pm$  SE.



**Figure 4.** Morphological analysis of (A) SLM-ERY-25 (magnifications of 4 $\times$ ) and (B) MLC-RSV-F (magnifications of 10 $\times$ ) by Microstar 110 optical microscope; photographs were taken manually by an external camera.

The bulk properties of the microparticles were evaluated in terms of theoretical and experimental flowability. The bulk and tapped volumes and densities were measured to calculate the compressibility index and the Hausner ratio, which—according to the European Pharmacopoeia—are indicative of powder flow characteristics (see Table S1, Supplementary Materials). As reported in Table 3, all SLM-ERY formulations exhibited similar behaviors, predictive of excellent flow properties, whereas MLC-RSV appeared highly cohesive, displaying very poor flow properties. These theoretical predictions were experimentally confirmed through angle-of-repose measurements. SLM-ERY formulations exhibited an angle of repose of approximately 25°, consistent with excellent flow characteristics. In contrast, MLC-RSV failed to flow under any tested conditions. Despite multiple operational adjustments, including variations in the funnel orifice diameter, no powder flow was observed, making it impossible to determine the angle of repose.

**Table 3.** Bulk and tapped densities ( $n = 3$ ), compressibility index % ( $n = 3$ ), Hausner ratio ( $n = 3$ ), and angle of repose ( $n = 6$ ) for the SLM-ERY and MLC-RSV-F. Means  $\pm$  SE.

| Parameter                      | SLM-ERY-10        | SLM-ERY-15        | SLM-ERY-25        | MLC-RSV-F          |
|--------------------------------|-------------------|-------------------|-------------------|--------------------|
| Bulk density (g/mL)            | 0.541 $\pm$ 0.006 | 0.534 $\pm$ 0.002 | 0.525 $\pm$ 0.002 | 0.328 $\pm$ 0.003  |
| Tapped density (g/mL)          | 0.582 $\pm$ 0.006 | 0.572 $\pm$ 0.002 | 0.566 $\pm$ 0.001 | 0.500 $\pm$ 0.005  |
| Compressibility index %        | 7.009 $\pm$ 0.002 | 7.189 $\pm$ 0.003 | 7.246 $\pm$ 0.004 | 34.399 $\pm$ 0.032 |
| Hausner ratio                  | 1.007 $\pm$ 0.002 | 1.007 $\pm$ 0.001 | 1.007 $\pm$ 0.007 | 1.520 $\pm$ 0.001  |
| Angle of repose ( $^{\circ}$ ) | 25.47 $\pm$ 0.21  | 25.64 $\pm$ 0.05  | 25.59 $\pm$ 0.07  | N.A.               |

Overall, SLM-ERY microparticles can be considered excellent pharmaceutical powders—homogeneous, reproducible, and free-flowing, with suitable softening and melting temperatures. MLC-RSV shared most of these features, with the exception of flowability.

However, the direct oromucosal administration of these powders is not straightforward, as they may exhibit limited wettability and mucoadhesiveness. This could lead to their rapid removal by salivary washout and subsequent swallowing, thereby reducing their residence time on the mucosal surface and ultimately compromising therapeutic efficacy. Furthermore, the administration of a reproducible dose of microparticles—and hence, the actives—would be difficult to achieve, especially for the non-flowing MLC-RSV. Hence, the microparticles represent promising intermediates for the development of solid formulations optimized for oromucosal delivery, such as mucoadhesive buccal patches. Given that increasing the ERY content did not markedly alter powder properties, SLM-ERY-25 was chosen for patch preparation to achieve higher drug loading with fewer microspheres.

## 2.2. Buccal Patches Preparation and Characterization

Mucoadhesive buccal patches were identified as the most appropriate formulation approach, combining the benefits of the lipid-based microparticles with the flexibility and mucoadhesive properties of hydrophilic polymeric films, to achieve effective oromucosal delivery. This strategy is expected to facilitate targeted accumulation of RSV and/or ERY at the gingival and buccal mucosa, thereby overcoming the limitations of systemic post-operative treatments and filling the current gap resulting from the lack of pharmaceutical dosage forms containing antibiotics specifically designed for locally acting antibiotic formulations for the oral cavity. For the preparation of the buccal patches, MLC-RSV-F and/or SLM-ERY-25 were homogeneously incorporated into a complex polymeric network, initially formulated as an aqueous gel, that, upon dehydration, formed a solid, dry, and flexible matrix. The solvent casting technique was selected to dry the microparticle suspension, as it offers a simple, scalable, cost-effective, and environmentally friendly method—particularly when aqueous solutions are employed as the casting medium. The polymeric gel, formulated according to previous work [18], consisted of hydroxyethyl cellulose (HEC), polyvinylpyrrolidone K30 (PVP K30), trehalose, and potassium sorbate in water. However, after oven-drying, the resulting patches were structurally fragile and prone to fracture. In addition, the ERY + RSV-patch<sub>DW</sub>, containing both SLM-ERY and MLC-RSV, exhibited an abnormal brownish discoloration (see Figure S1, Supplementary Materials). This color alteration suggested possible RSV degradation, consistent with its known instability under certain conditions, particularly in alkaline environments. This hypothesis was confirmed by RSV quantification, which showed levels markedly below the theoretical value. To verify whether this degradation was associated with an unsuitable pH, measurements were performed on the base gel, gels containing MLC-RSV and/or SLM-ERY, and the resulting patch discs after immersion in both distilled water and artificial saliva at pH 6.8. As reported in Table 4, none of the measured pH values fell within the range required to maintain RSV stability [22]. Notably, the dispersion of SLM-ERY in the gel led to a marked pH increase,

suggesting that a portion of ERY was present on the microparticle surface. Consequently, upon dispersion in the aqueous medium, ERY—being a weak base—raised the pH of the formulation. When MLC-RSV was subsequently incorporated, it was unable to prevent RSV degradation during drying, which thus occurred under alkaline conditions (30 °C for 48 h). In addition to compromising RSV stability, an alkaline pH is undesirable for topical application to damaged tissues, as mildly acidic conditions are known to promote wound healing [23]. Based on these findings, optimization of the formulation pH was deemed necessary. Therefore, deionized water was replaced with citrate buffers (CB1–4, [24]) of varying pH and ionic strength. Accordingly, pH measurements were repeated at each step of the patch preparation process and after patch formation (Table 4).

**Table 4.** pH values of preparation intermediates (pure solvent, gel, gel + SLM-ERY, and gel + SLM-ERY + MLC-RSV) and patches (kept in water or artificial saliva). Means ( $n = 3$ )  $\pm$  SE.

| Sample                                                               | DW              | CB1             | CB2             | CB3              | CB4             |
|----------------------------------------------------------------------|-----------------|-----------------|-----------------|------------------|-----------------|
| <b>Solvent</b>                                                       | 7.00 $\pm$ 0.00 | 5.14 $\pm$ 0.02 | 4.41 $\pm$ 0.01 | 5.10 $\pm$ 0.01  | 4.40 $\pm$ 0.01 |
| <b>Gel<sub>solvent</sub></b>                                         | 7.11 $\pm$ 0.17 | 5.55 $\pm$ 0.04 | 4.74 $\pm$ 0.06 | 5.32 $\pm$ 0.03  | 4.56 $\pm$ 0.02 |
| <b>Gel<sub>solvent</sub> + SLM-ERY</b>                               | 8.56 $\pm$ 0.09 | 6.49 $\pm$ 0.05 | 4.84 $\pm$ 0.02 | 5.68 $\pm$ 0.03  | 4.85 $\pm$ 0.02 |
| <b>Gel<sub>solvent</sub> + SLM-ERY + MLC-RSV</b>                     | 8.48 $\pm$ 0.04 | 5.97 $\pm$ 0.06 | 5.00 $\pm$ 0.02 | 5.69 $\pm$ 0.02  | 5.20 $\pm$ 0.03 |
| <b>ERY-patch<sub>solvent</sub></b> <i>in water</i>                   | 8.92 $\pm$ 0.04 | 7.94 $\pm$ 0.01 | 7.04 $\pm$ 0.01 | 6.67 $\pm$ 0.003 | 5.51 $\pm$ 0.01 |
| <b>ERY-patch<sub>solvent</sub></b> <i>in artificial saliva</i>       | 8.20 $\pm$ 0.02 | 7.60 $\pm$ 0.02 | 7.31 $\pm$ 0.02 | 7.10 $\pm$ 0.01  | 6.47 $\pm$ 0.02 |
| <b>ERY + RSV-patch<sub>solvent</sub></b> <i>in water</i>             | 8.79 $\pm$ 0.02 | 8.25 $\pm$ 0.02 | 6.50 $\pm$ 0.01 | 6.55 $\pm$ 0.01  | 5.60 $\pm$ 0.02 |
| <b>ERY + RSV-patch<sub>solvent</sub></b> <i>in artificial saliva</i> | 8.08 $\pm$ 0.01 | 7.57 $\pm$ 0.02 | 7.13 $\pm$ 0.01 | 7.14 $\pm$ 0.01  | 6.51 $\pm$ 0.01 |

As reported, CB1 (pH 5.5; 13 mM) was not sufficient to maintain the desired pH ( $\leq 5.5$ ) after microparticle mixing, leading to slightly colored patches due to RSV instability (see Figure S1, Supplementary Materials). CB3 (pH 4.4; 16 mM) and CB4 (pH 4.4; 32 mM) allowed us to obtain the best pH values for the gel–microparticle intermediates. However, both patches obtained with CB3 (pH 5.0; 25 mM) and CB4 were found to be mechanically fragile (see Figure S1, Supplementary Materials), likely due to the high amount of salt crystallized within the matrix during drying, which caused rigidity and brittleness. This was confirmed by folding endurance values of 2 for CB3- and CB4-based patches, compared with values  $> 300$  for ERY-patches<sub>CB2</sub> and ERY + RSV-patches<sub>CB2</sub>, which demonstrated excellent flexibility. Such mechanical weakness is unsuitable for oromucosal applications, where flexibility is required to ensure proper adhesion and resistance to oral movements, and only ERY-patches<sub>CB2</sub> and ERY + RSV-patches<sub>CB2</sub> formulations were selected for subsequent investigations.

To validate the preparation technique, the resulting patches were characterized. Measurements of weight and residual water content (%) (Table 5) confirmed the reproducibility of the manufacturing process. Notably, a small amount of water was retained within the polymeric matrix after drying, likely contributing to the flexibility of the final formulation. Owing to this residual moisture, potassium sorbate was included as a preservative in the subsequent batches of patches to ensure microbiological stability during storage. Patch uniformity was assessed in terms of weight, thickness, and RSV and/or ERY content, expressed both as the dose per unit area and Drug Loading % (DL%). The reported results (Table 5) confirmed the homogeneity of the buccal delivery systems obtained—fundamental aspects for the reliability and effectiveness of formulations—to guarantee a controlled and consistent dosage for each unit dose. Indeed, within the framework of a personalized therapeutic approach, a 20.25 cm<sup>2</sup> patch can be easily cut into smaller portions to match the size of the treatment site and deliver the desired drug dose.

**Table 5.** Buccal patches characterization: weight of the entire patch ( $n = 5$ ); uniformity in terms of weight ( $n = 15$ ), thickness ( $n = 25$ ), microparticles concentration ( $n = 5$ ), and active(s) content ( $n = 15$ ) of patch portions ( $0.52 \text{ cm}^2$  discs). Means  $\pm$  SE.

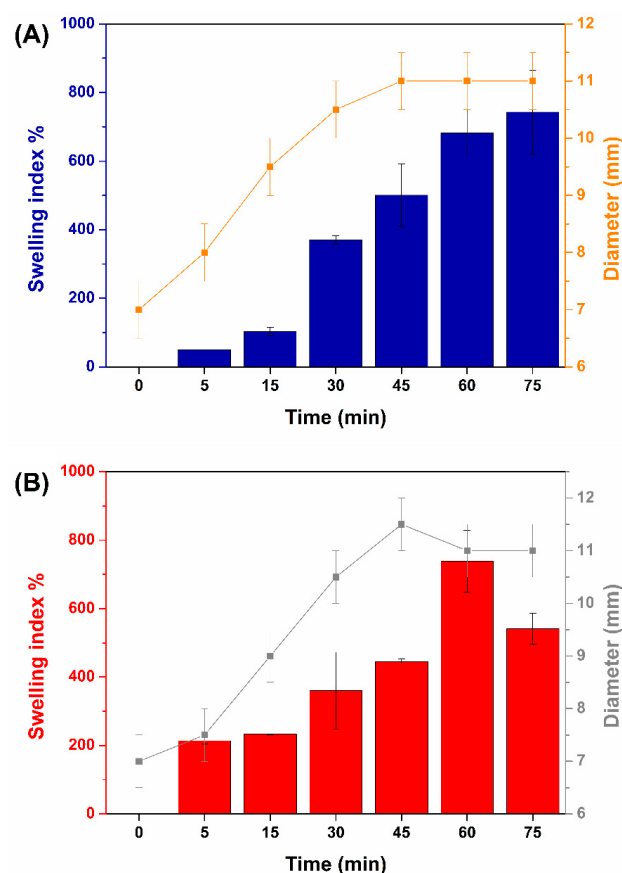
|                                           |                          | ERY-Patch <sub>CB2</sub> | ERY + RSV-Patch <sub>CB2</sub> |
|-------------------------------------------|--------------------------|--------------------------|--------------------------------|
| <b>Weight<sub>patch</sub> (mg)</b>        |                          | 524.7 $\pm$ 1.9          | 614.9 $\pm$ 4.5                |
| <b>Residual water content %</b>           |                          | 5.06 $\pm$ 0.34          | 4.04 $\pm$ 0.45                |
| <b>Weight<sub>disc</sub> (mg)</b>         |                          | 13.93 $\pm$ 0.56         | 18.76 $\pm$ 0.55               |
| <b>Thickness (mm)</b>                     |                          | 0.647 $\pm$ 0.024        | 0.630 $\pm$ 0.007              |
| <b>Microparticles concentration (w/w)</b> |                          | 60.99 $\pm$ 0.13         | 52.04 $\pm$ 0.22               |
| <b>ERY content</b>                        | <b>mg/cm<sup>2</sup></b> | 3.60 $\pm$ 0.08          | 3.70 $\pm$ 0.17                |
|                                           | <b>DL% (w/w)</b>         | 13.45 $\pm$ 0.31         | 11.09 $\pm$ 0.26               |
| <b>RSV content</b>                        | <b>mg/cm<sup>2</sup></b> | /                        | 0.31 $\pm$ 0.02                |
|                                           | <b>DL% (w/w)</b>         | /                        | 0.92 $\pm$ 0.04                |

An additional parameter of interest was the swelling index (SI%) of the patches. As the system consists of polymers prone to hydration and expansion in aqueous environments, the swelling behavior was evaluated from multiple perspectives: (i) weight gain, and (ii) dimensional changes, including both radial (diameter) and axial (thickness) swelling, upon wetting with artificial saliva. The SI% was measured at regular intervals for up to 90 min, allowing a preliminary estimation of the maximum residence time of the patches on the mucosa before the polymeric film began to disintegrate. Both formulations began to dissolve after approximately 60 min and were completely disintegrated by 90 min (see Figures S2 and S3, Supplementary Materials). This timeframe represents an ideal residence period for mucoadhesive systems, being sufficiently long to ensure effective release of the active ingredient(s) while avoiding excessive persistence that could cause discomfort or irritation. Figure 5 reports the SI% and temporal changes in patch diameter, both of which provide valuable insight into the system's performance. Regarding the SI% values, both types of patches exhibited a high water uptake capacity, showing an approximately eightfold increase in weight relative to their initial mass. Such behavior is advantageous, as water absorption and the consequent expansion of the hydrophilic matrix facilitate the release of entrapped microparticles, promoting their migration to the mucosal surface. There, they may soften or fuse, enhancing tissue penetration and acting as a permeation enhancer. Although a high SI favors efficient microparticle release, the resulting volume increase must not compromise formulation tolerability. Excessive thickness or diameter enlargement could cause discomfort and promote patch displacement due to involuntary tongue movements. Fortunately, no increase in thickness was observed following water uptake, whereas radial swelling progressed for approximately 50 min before reaching a plateau, with the final diameter being about 1.5 times the initial value (Figure 5). This moderate expansion may enhance mucosal coverage and active ingredient distribution without compromising adhesion (see Figures S2 and S3, Supplementary Material).

### 2.3. Ex Vivo Mucoadhesion Studies

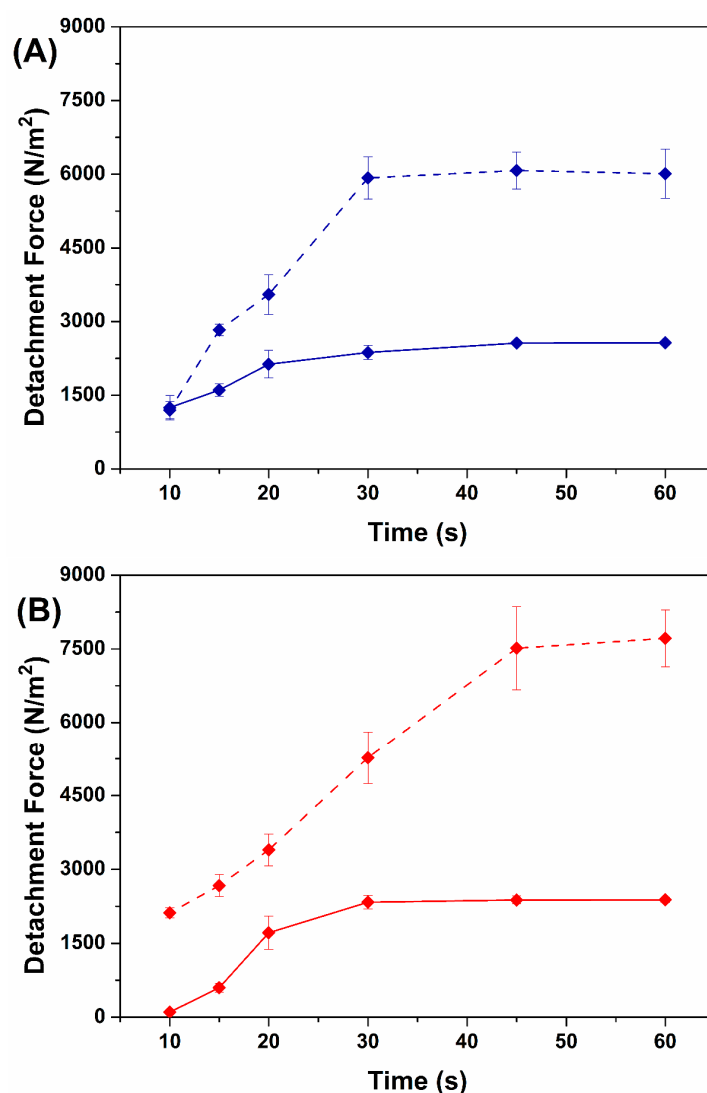
Mucoadhesion is also a critical parameter for ensuring the therapeutic efficacy of the patches, as it determines their ability to adhere firmly to the mucosal surface for a sufficient duration, allowing effective contact between the microparticles and the mucosa. Mucoadhesion was assessed both qualitatively and quantitatively. In the qualitative ex vivo study, patch portions ( $2 \times 1 \text{ cm}$  in size) were applied to porcine buccal tissues pretreated with artificial saliva at physiological temperature. As shown in Figure S4 (Supplementary Material), the patch adhered completely to the mucosal surface and maintained adhesion under mechanical stresses of the tissue (such as rotation, bending, and inversion). The formulation also exhibited elastic adaptability to tissue movements, confirming its flexibility, consistent

with folding endurance results, and supporting the mechanical strength and ductility of the polymeric matrix during application.



**Figure 5.** Swelling/disintegration assay showing the swelling index (bars) and diameter (squares) for ERY-patch<sub>CB2</sub> (A) and ERY + RSV-patch<sub>CB2</sub> (B).

Furthermore, using a texture analyzer, it was possible to obtain information regarding the force and/or work required to detach the dosage form from a simulated or ex vivo mucosal membrane [25]. This type of analysis provides various insights into the characteristics of the dosage form under investigation since the force of adhesion and the work of adhesion describe different physical phenomena. Specifically, the force of adhesion—usually normalized to the contact surface area to obtain the detachment force—is a directly measured parameter that reflects the force required to separate the two surfaces after contact and the establishment of a mucoadhesive interaction, thereby indicating the instantaneous resistance to detachment. Considering the complexity of the mucoadhesion phenomenon, it is crucial to consider the initial contact time between the formulation and the mucosa, as this parameter plays a key role in the formation and stabilization of bonds between them. For this purpose, the mucoadhesive behavior was evaluated as a function of the starting contact time before applying traction (Figure 6). Moreover, it should be considered that the ex vivo tissues used were washed and deprived of their mucous layer and the mucins naturally present in the oral cavity. Since mucins play a key role in mucoadhesion, the experiments were conducted both in the absence (continuous lines in Figure 6) and presence (dashed lines in Figure 6) of deliberately added mucins [26–29].



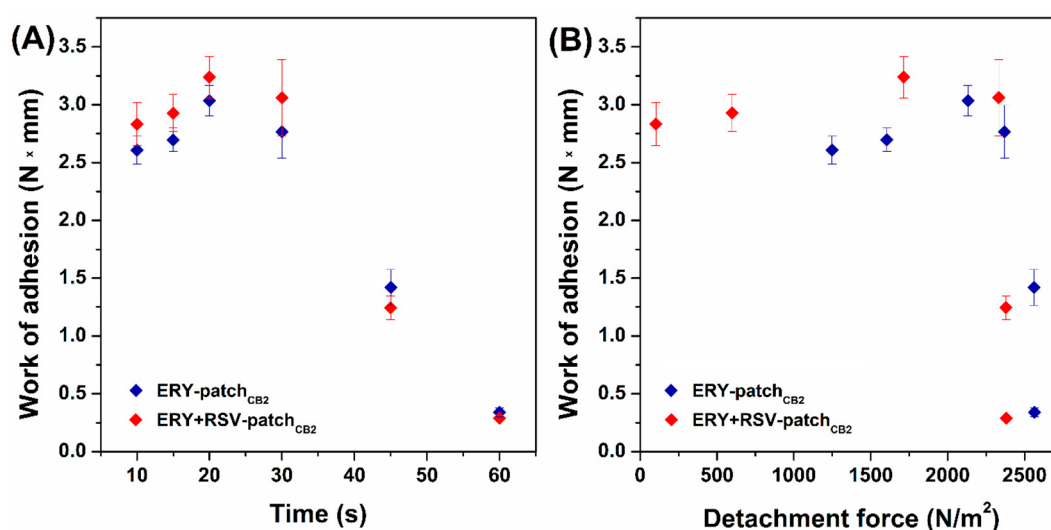
**Figure 6.** Ex vivo quantitative mucoadhesion profiles showing detachment force (N/m<sup>2</sup>) versus time for the ERY-patch<sub>CB2</sub> (A) and the ERY + RSV-patch<sub>CB2</sub> (B) recorded in the absence (solid line) or presence (dashed line) of mucins.

As reported, the detachment force increased as a function of the initial contact time until a plateau was reached. Additionally, for short contact times, the ERY-patch<sub>CB2</sub> formulation exhibited a higher adhesion force than the ERY + RSV-patch<sub>CB2</sub> formulation, whereas for contact times  $\geq 30$  s, the two patch types displayed comparable behavior in the absence of mucins. These differences may be attributed to the distinct compositions of the patches. In fact, the ERY-patch<sub>CB2</sub>, containing only SLM-ERY, features a hydrophilic matrix-to-microparticles ratio of 1:1.77, while the ERY + RSV-patch<sub>CB2</sub>, which contains both SLM-ERY and MLC-RSV, presents a ratio of 1:2.33. These compositional differences affect the initial behavior because, for an equivalent surface area, ERY-patch<sub>CB2</sub> contains a greater proportion of hydrophilic matrix capable of forming additional interactions with the tissue, resulting in a higher detachment force. However, at longer contact times, such small quantitative differences in matrix content become negligible, while other factors may be relevant. Moreover, for polymeric matrices that did not dissolve over the timeframe considered, a relationship can be observed between the swelling capacity and mucoadhesion, which is enhanced by the increased surface contact area resulting from swelling. The water uptake may also initially contribute to reducing the cohesive interactions within the

formulation, thereby making its components available for the formation of interactions with the mucosal tissue [25,30].

Although the literature does not provide universally accepted threshold values to define the degree of mucoadhesion—as these depend on parameters such as the equipment used, contact force, contact time between the film and the substrate, traction speed, and film sample area [31]—indicative ranges could nonetheless be considered: detachment forces between 200 and 1000 N/m<sup>2</sup> are generally associated with moderate mucoadhesion [32], whereas values exceeding 1000 N/m<sup>2</sup> indicate strong mucoadhesion [33,34]. The patches developed in the present study exhibited detachment force values of about 2500 N/m<sup>2</sup>, thereby confirming their high adhesive capacity—a key feature for the efficacy of oromucosal delivery systems. Moreover, a significant 2- to 4-fold increase in the detachment force was observed in the presence of mucins. These results are particularly relevant for the intended application, as a contact time of up to 60 s reflects the kinetics of interfacial bond formation between the polymeric matrix and the mucosa and represents a practical duration. Longer contact times would be unrealistic and potentially uncomfortable for the patient, who would otherwise need to apply continuous manual pressure on the patch for an extended period, leading to discomfort and pain.

Another parameter frequently considered in mucoadhesion studies is the work of adhesion, corresponding to the hysteresis area of the force–distance curves. Since the most commonly used models of mucoadhesion (e.g., the JKR model) describe ideal adhesion between two elastic solids, they do not account for the existence of this hysteresis area. However, considering that the biological mucosae and the biopolymers used for the hydrophilic base of the patches are characterized by deformability and, more specifically, by viscoelastic rheological behavior—which is known to promote the mucoadhesive behavior [35]—their interaction led to an irreversible dissipation of energy during the adhesion–detachment cycle, which is quantified as the work of adhesion. This parameter is especially relevant for the patches studied, which cannot be considered purely elastic solids owing to their chemical heterogeneity (a hydrophilic polymeric matrix containing lipid microparticles), deformability, surface roughness (due to microparticles), and swelling capacity [36]. Indeed, experimentally, the presence of a hysteresis area was consistently observed, and the relationship between the work of adhesion and contact time (Figure 7A) or detachment force (Figure 7B) was investigated.



**Figure 7.** Work of adhesion (N·mm) versus (A) time (s) or (B) detachment force (N/m<sup>2</sup>) for the ERY-patch<sub>CB2</sub> (blue) and the ERY + RSV-patch<sub>CB2</sub> (red) recorded in the absence of mucins as representative examples.

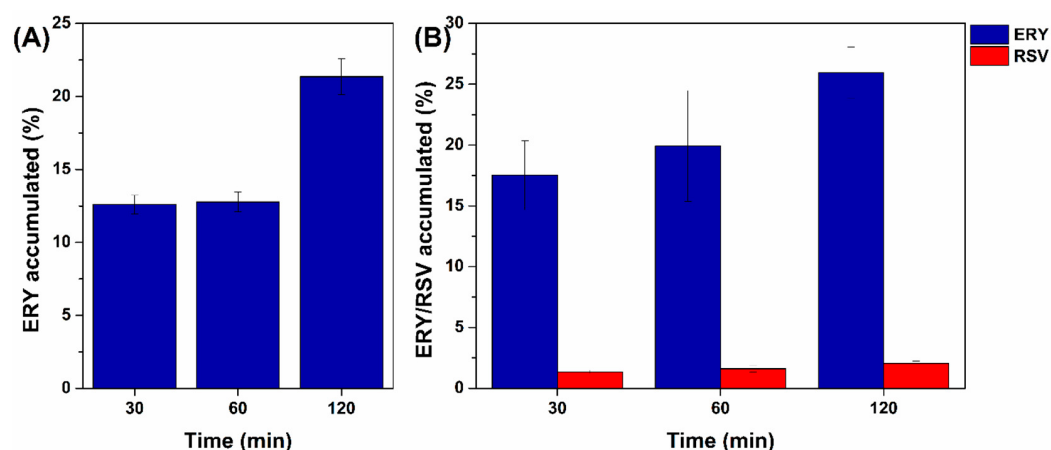
Both patches exhibited very similar behavior. The work of adhesion did not vary significantly at short contact times (<30 s), whereas it underwent a marked, approximately linear decrease with increasing initial contact durations. This sharp reduction coincides with the attainment of the plateau in terms of the detachment force. To summarize, after 30 s of contact at a constant applied force, the system can be considered to have reached its maximum instantaneous adhesive efficiency, meaning that the formulation–tissue interactions have achieved equilibrium. At the same time, the formulation becomes hydrated and begins to swell—a process that, over time, will also contribute to its dissolution—thus becoming less cohesive. As a result, during detachment, the formulation exhibited less prolonged resistance, leading to a lower energy dissipation. In fact, considering that the dominant mechanism of energy dissipation is likely the rupture of weak physical interactions formed between the formulation and the mucosal tissue, the interposition of water molecules between these two phases contributes to minimizing the work of adhesion [37]. It is also relevant to note that the results obtained when soaking the mucosal surface in a mucin dispersion revealed behaviors analogous to those shown in Figure 7, both in quantitative terms (hysteresis areas) and in the overall trend of the curves. Consequently, the presence or absence of mucins on the tissue surface appeared to affect only the instantaneous detachment force, while it did not affect the work of adhesion, which was therefore predominantly related to the viscoelastic properties of the materials under investigation—properties that remained constant across the two experimental sets.

#### 2.4. *Ex Vivo* Permeation/Penetration of ERY and RSV Following Buccal Patch Application

Finally, *ex vivo* permeation studies were conducted to assess the ability of the active(s) to penetrate and/or permeate the buccal mucosa. The experiments were stopped at three different time points (30, 60, and 120 min) based on the swelling test results, indicating that the patches dissolved after approximately 90 min. Extending the duration beyond 120 min would not accurately reproduce physiological conditions, as *in vivo*, the dissolved film would be progressively removed by salivary washout and subsequent swallowing, whereas the *in vitro* static setup would not account for these clearance mechanisms, thereby yielding data unrepresentative of *in vivo* behavior. As a result, neither ERY nor RSV was detected in the acceptor chamber before 120 min, confirming the absence of transmucosal absorption of the two molecules. This finding supports the therapeutic goal of providing a localized effect at the site of application while avoiding systemic side effects. Accordingly, to assess tissue retention, the mucosal membranes were rinsed at each time point and subsequently extracted to quantify the fraction of active(s) accumulated within the tissue. The results (Figure 8) indicated a progressive accumulation of both ERY and RSV, with slightly higher ERY retention when administering the ERY + RSV-patch<sub>CB2</sub> compared with the ERY-patch<sub>CB2</sub>. This difference might be attributed to the presence of Labrasol® as the liquid component of the MLC-RSV. Indeed, Labrasol® is a mixture containing a small fraction of mono-, di-, and triglycerides and PEG-8 (MW 400), both esterified with mono- and diesters of caprylic (C8) and capric (C10) acids and in free form. This amphiphilic nature of the compound confers solubilizing and penetration-enhancing properties [19], thereby promoting ERY accumulation.

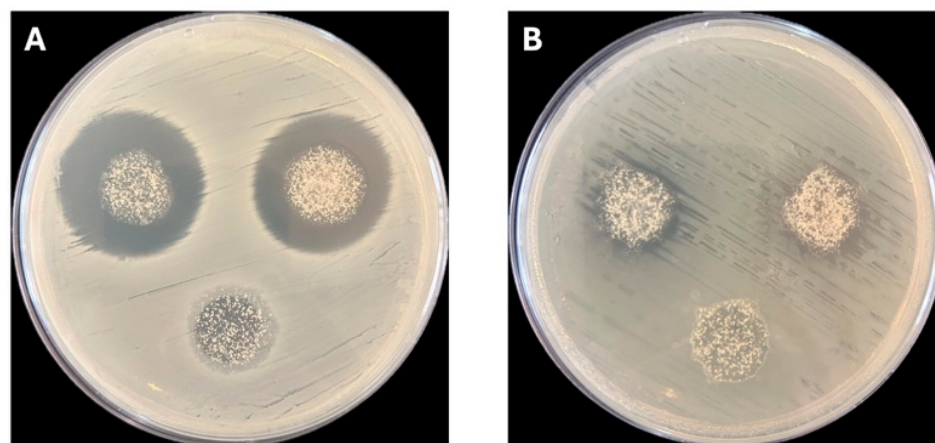
Consequently, the aim of delivering the selected antibiotic directly at the mucosal site was fully achieved, with approximately 21% and 26% of the initial ERY dose retained in the tissue after 120 min. When an ERY + RSV-patch<sub>CB2</sub> was administered, roughly 2% of the initial RSV dose was also entrapped in the buccal mucosa. As RSV exerts its biological effects at low doses, its inclusion as a coadjuvant—with osteoinductive, antioxidant, and wound-healing properties [12,13,38]—could further enhance the overall efficacy of the formulation, supporting both surgical site asepsis and tissue and bone regeneration. The

low RSV retention, compared with the ERY results, was considered acceptable, as it is well known that RSV, like other polyphenols, exhibits antioxidant and wound-healing activities even at extremely low doses. Furthermore, it should be emphasized that at higher doses, these compounds may also exert chemopreventive and anticancer effects [39]. It is relevant to point out that after 120 min of the ex vivo assay, both patches appeared swollen, being more like a highly viscous gel, and completely adhered to the mucosal surface. The latter is in accordance with the swelling and disintegration data.



**Figure 8.** Percentage of ERY and RSV (when present) retained in the buccal mucosa after 15, 30, or 60 min following administration of ERY-patch<sub>CB2</sub> (A) and ERY + RSV-patch<sub>CB2</sub> (B).

Finally, to verify the antimicrobial action of the proposed formulation, as well as to further prove the release of the active(s), thus supporting the proposed clinical application, the antimicrobial activity of both formulations was tested by patch-on-agar diffusion studies. The assay revealed clear, strain-dependent differences in antibacterial susceptibility, as evidenced by the inhibition halos surrounding the antibiotic-loaded patches (Figure 9).



**Figure 9.** Antibacterial activity assay against *Staphylococcus aureus* ATCC 25923 (A) and *Escherichia coli* ATCC 25922 (B). In both panels, the top-left sample is the ERY-patch<sub>CB2</sub>, the top-right sample is the ERY + RSV-patch<sub>CB2</sub>, and the bottom sample is the empty control patch.

In Figure 9A, the *Staphylococcus aureus* ATCC 25923 strain displayed well-defined and reproducible inhibition halos around both the ERY-patch<sub>CB2</sub> (top left) and the ERY + RSV-patch<sub>CB2</sub> (top right). The analysis performed by repeating the experiment on three independent patches for each condition showed average inhibition halo diameters of approximately  $30 \text{ mm} \pm 1 \text{ mm}$  for both patches. These results indicated a strong susceptibility of this Gram-positive strain to ERY and demonstrated that the incorporation of RSV did not

adversely affect antibiotic diffusion or activity, while preserving a robust antibacterial effect. As expected, no inhibition zone is detected around the empty patch (bottom), confirming the absence of intrinsic antibacterial activity of both the microparticle components and the patch matrix. Instead, the *Escherichia coli* ATCC 25922 strain (Figure 9B) showed inhibition halos noticeably smaller and less sharply defined than those observed for *S. aureus*. This behavior is consistent with the intrinsic reduced susceptibility of Gram-negative bacteria to macrolides, largely attributable to the presence of the outer membrane, which limits antibiotic penetration [40,41]. Due to the diffuse and poorly defined nature of these halos, it was not possible to reliably measure the inhibition zone diameter for either of the formulations. No growth inhibition is observed around the empty patch, further confirming that the antibacterial activity is specifically associated with the presence of ERY.

The obtained results further confirm the actual release of the active(s) from the microparticles, as early evidenced by the ex vivo studies.

### 3. Materials and Methods

#### 3.1. Materials

Erythromycin (ERY) was purchased from Galeno (Carmignano, PO, Italy). Trans-Resveratrol (RSV), medium-viscosity hydroxyethylcellulose (HEC),  $\beta$ -cyclodextrins ( $\beta$ -CD), and polyvinylpyrrolidone K30 (PVP K30) were purchased from A.C.E.F. (Fiorenzuola D'Arda, PC, Italy). Labrasol® (PEG-8 caprylic/capric glycerides) was kindly supplied by Gattefossé (Saint-Priest, France). 1-Hexadecanol and mucin from porcine stomach type III were obtained from Sigma-Aldrich (Steinheim, Germany). Cetyl decanoate was synthesized according to the previously reported procedure [18]. Lemon essential oil was purchased from Farmalabor (Canosa di Puglia, Italy). Potassium sorbate was obtained from Carlo Erba Reagents S.r.l. (Milan, Italy). Trifluoroacetic acid was purchased from Merck (Darmstadt, Germany). Trehalose was purchased from Hayashibara Shoji Inc. (Okayama, Japan). The DPPH reagent (2,2-diphenyl-1-picrylhydrazyl radical) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). The following aqueous solutions were prepared: isotonic solution, containing 9.0 g of NaCl in 1 L of distilled water, and the same containing trehalose (5% *w/v*); highly concentrated NaCl solution, containing 350 g of NaCl in 1 L of distilled water. Citrate buffer solutions were prepared using different ratios of sodium citrate dihydrate and citric acid monohydrate at various molarities and pH values: Citrate buffer pH 5.5—13 mM (CB1); Citrate buffer pH 4.4—16 mM (CB2); Citrate buffer pH 5.0—25 mM (CB3); Citrate buffer pH 4.4—32 mM (CB4); Citrate buffer CB1 containing also  $\beta$ -cyclodextrins (3% *w/v*). Non-enzymatic artificial saliva at pH 6.8 was prepared by dissolving 0.126 g of NaCl, 0.937 g of KCl, 0.189 g of KSCN, 0.655 g of  $\text{KH}_2\text{PO}_4$ , 0.200 g of urea, 0.154 g of  $\text{Na}_2\text{SO}_4$ , 0.178 g of  $\text{NH}_4\text{Cl}$ , 0.130 g of  $\text{CaCl}_2$ , and 0.631 g of  $\text{NaHCO}_3$  in 1 L of distilled water. A second dispersion was prepared by supplementing the above solution with 1% (*w/v*) mucin from porcine stomach, type III. All solvents and salts were used without further purification and were purchased from Carlo Erba Reagents S.r.l. (Milan, Italy) and VWR International S.r.l. (Milan, Italy). Porcine buccal mucosa was obtained from tissues derived from animals intended for human consumption, kindly supplied by Azienda Agricola Mulinello S.r.l. (Enna, Italy). Owing to their origin, their use does not require approval by the ethics committee.

#### 3.2. Preparation of Erythromycin-Loaded Solid Lipid Microparticles (SLM-ERY)

The SLM-ERY formulations were prepared using the hot melt dispersion technique according to the procedure described in Italian Patent No. ITRM102019000011436. Precisely weighed amounts of ERY, 1-Hexadecanol, and Cetyl decanoate were placed in a 400 mL beaker and melted on a heating plate (Heidolph MR3001K Hotplate Stirrer with Heidolph

EXT3001 Temperature Probe, Heidolph Instruments, Schwabach, Germany). When present, 15 µL of lemon essential oil was added to the molten lipid mixture. In parallel, 75 mL of a highly concentrated NaCl solution was brought to the boil and subsequently added to the molten lipid phase. The resulting biphasic system was subjected to constant mechanical stirring using a bladed stirrer (RW 20 S12, Kinematica, Malters, Switzerland) at 1500 rpm for 1 min. Thereafter, the system, maintained under the same stirring conditions, was externally cooled in an ice-water bath to promote microparticle solidification, prolonging agitation for 7 min. The solid lipid microparticles thus obtained were separated by flotation and filtration, rapidly washed on the filter with distilled water, and then left to dry on filter paper at room temperature for 48 h. Batches of 2 g microparticles were prepared. For each of the three lipid mixtures reported in Table 6, five independent batches of microparticles were produced.

**Table 6.** Percentage composition of the lipid mixtures employed to prepare SLM-ERY.

| Formula Code | 1-Hexadecanol (%) | Cetyl Decanoate (%) | Lemon Essential Oil (µL) | Erythromycin (%) |
|--------------|-------------------|---------------------|--------------------------|------------------|
| SLM-ERY-10   | 65.00             | 25.00               | /                        | 10.00            |
| SLM-ERY-15   | 60.00             | 25.00               | 15                       | 15.00            |
| SLM-ERY-25   | 52.95             | 22.05               | 15                       | 25.00            |

The yield % of the process was calculated as follows:

$$\text{Yield \%} = \frac{\text{Recovered SLM} - \text{ERY (g)}}{\text{Starting lipid mixture (g)}} \times 100$$

The results are reported as the mean ( $n = 5$ )  $\pm$  standard error (SE).

### 3.3. Preparation of Resveratrol-Loaded Microstructured Lipid Carriers (MLC-RSV)

MLC-RSV was prepared according to the previously described SLM-ERY preparation technique. However, in this case, it was necessary to first dissolve RSV in Labrasol® at  $100.0 \pm 0.5$  °C and subsequently add the other lipid components until each of them was completely melted, while carefully avoiding any darkening of the mixture, indicative of RSV degradation. Then, 10 µL of lemon essential oil was added to the molten lipid mixture. Afterward, 75 mL of a boiling, highly concentrated NaCl solution was added to the molten lipid mixture, and the resulting biphasic system was subjected to 700 rpm mechanical stirring for 1 min. Thereafter, the system was externally cooled in an ice-water bath to promote microparticle solidification, prolonging agitation for 6 min. The solid lipid microparticles thus obtained were separated by flotation and filtration, rapidly washed with distilled water, and then left to dry on filter paper at room temperature for 48 h. Batches of 3 g microparticles were prepared. For each of the six lipid mixtures reported in Table 7, five independent batches of microparticles were produced. The yield % of the process was calculated as previously described, and the results are reported as the mean ( $n = 5$ )  $\pm$  SE.

**Table 7.** Percentage composition of the lipid mixtures employed to prepare MLC-RSV.

| Formula Code | Labrasol® (%) | 1-Hexadecanol (%) | Cetyl Decanoate (%) | Lemon Essential Oil (µL) | Resveratrol (%) |
|--------------|---------------|-------------------|---------------------|--------------------------|-----------------|
| MLC-RSV-A    | 52.25         | 42.75             | /                   | 10                       | 5.00            |
| MLC-RSV-B    | 57.00         | 38.00             | /                   | 10                       | 5.00            |
| MLC-RSV-C    | 61.75         | 33.25             | /                   | 10                       | 5.00            |
| MLC-RSV-D    | 47.50         | 38.00             | 9.50                | 10                       | 5.00            |
| MLC-RSV-E    | 47.50         | 36.10             | 11.40               | 10                       | 5.00            |
| MLC-RSV-F    | 44.65         | 36.10             | 14.25               | 10                       | 5.00            |

### 3.4. Evaluation of Particles' Melting Temperature Range

Small amounts from each batch of SLM-ERY or MLC-RSV were placed into a glass capillary, which was inserted into a Stuart melting point apparatus (SMP30, Bibby Scientific Ltd., Staffordshire, UK). The instrument was set at a starting temperature of 26 °C and programmed to increase at a rate of 1.5 °C/min. The softening temperature range and melting point were recorded at the onset of particle softening and upon the formation of a clear solution, respectively. Each analysis was performed in duplicate for every batch, and results are reported as the mean ( $n = 10$ )  $\pm$  SE.

### 3.5. DL% and LE% Evaluation

To quantify the ERY and RSV loaded in microparticles, HPLC-DAD and UV-Vis analyses were performed, respectively. The HPLC-DAD analysis was conducted using an Agilent 1260 Infinity system equipped with a G1311B quaternary pump coupled to a 1260 Infinity II diode array detector and fitted with a G7129C autosampler with integrated column oven, controlled by automatic integration software OpenLAB CDS ChemStation Workstation, version C.01.10, (Agilent Technologies, Santa Clara, CA, USA). Samples were prepared by dissolving accurately weighed amounts (10 or 20 mg) of SLM-ERY in 5 mL of MeOH. Chromatographic separation was carried out by injecting 20  $\mu$ L of each sample, with chromatograms recorded at 220 nm (DAD detection range: 190–640 nm). The stationary phase consisted of a reverse-phase Ace<sup>®</sup> Excel Super C18 column (125  $\times$  4.60 mm; 100 Å; 5  $\mu$ m). The mobile phase consisted of 0.1% *v/v* trifluoroacetic acid in water (solvent A) and acetonitrile (solvent B), under the following gradient conditions: 0–2 min: isocratic A:B = 90:10; 2–15 min: gradient from A:B = 90:10 to A:B = 35:65; 15–18 min: gradient from A:B = 35:65 to A:B = 90:10; 18–20 min: isocratic A:B = 90:10. To quantify ERY, a calibration curve was constructed, analyzing six MeOH standard solutions of ERY within a concentration range of 0.025–2.000 mg/mL:  $\lambda_{\text{max}} = 220$  nm, retention time = 13.3 min, linear regression equation:  $\text{Area} = 773.68 \times C$  (mg/mL) ( $R = 0.999$ ; LOQ: 0.006 mg/mL; LOD: 0.002 mg/mL).

The UV-Vis analysis was performed using a Shimadzu PharmaSpec 1700 instrument (Shimadzu, Kyoto, Japan). Samples were prepared by dissolving accurately weighed amounts (10 mg) of MLC-RSV in 5 mL of MeOH and then diluting the resulting solution 1:5 with MeOH. To quantify RSV, a calibration curve was constructed, analyzing six MeOH standard solutions of ERY, within a concentration range of  $1 \times 10^{-5}$ –0.005 mg/mL:  $\lambda = 305$  nm, linear regression equation:  $\text{Abs} = 0.0135 + 121.62 \times C$  (mg/mL) ( $R = 0.999$ ; LOQ:  $8 \times 10^{-7}$  mg/mL; LOD:  $2 \times 10^{-7}$  mg/mL).

The drug loading % and loading efficacy % were calculated as follows:

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

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

Each analysis was performed in triplicate on each prepared batch, and the results are reported as means ( $n = 15$ )  $\pm$  SE.

### 3.6. DPPH Assay

The DPPH assay was performed to evaluate the maintenance of the scavenger activity of RSV when embedded into the MLC-RSV-F. The assay was carried out as previously reported [39]. Briefly, 2 mL of DPPH stock solution (0.040 mg/mL in MeOH) was transferred into a quartz cuvette and supplemented with 100  $\mu$ L of the sample, vigorously mixed, and immediately subjected to UV-Vis analysis. Measurements were repeated every

5 min up to 1 h. Test samples were (i) 4 mg/mL of MLC-RSV in MeOH; (ii) the control RSV solution at the same RSV concentration, considering the previously obtained DL% value (0.2 mg/mL in MeOH); and (iii) MeOH used as the reference blank for the calculation of the initial concentration of the DPPH radical at time zero. To determine the amount of residual DPPH radical at the chosen time points, a calibration curve was constructed by analyzing 6 DPPH standard solutions: concentration range: 0.004–0.040 mg/mL;  $\lambda = 515$  nm; linear regression:  $\text{Abs} = -0.0186 + 26.92 \times C$  (mg/mL) ( $R = 0.999$ ). The assay was performed in triplicate, and the results are expressed as the percentage of residual DPPH radical (mean  $\pm$  SE;  $n = 3$ ) as a function of time (min).

### 3.7. Differential Thermal Analysis (DTA)

DTA was performed using a Netzsch Jupiter F1 STA 449 instrument (NETZSCH B.V. & Co. Holding KG, Selb, Germany) over a temperature range of 20–1000 °C, at a heating rate of 10 °C min<sup>−1</sup>, and under a dynamic atmosphere consisting of nitrogen and air, each supplied at a flow rate of 20 mL min<sup>−1</sup>. The analyzed samples were ERY, 1-hexadecanol, cetyl decanoate, and SLM-ERY-25.

### 3.8. Differential Scanning Calorimetry (DSC)

DSC was carried out using a Setaram DSC131 EVO instrument (Caluire, France) over a temperature range of 20–300 °C, at a heating rate of 10 °C min<sup>−1</sup>, and under a continuous nitrogen flow of 1 mL min<sup>−1</sup>. The analyzed samples were RSV and MLC-RSV-F.

### 3.9. Individual Properties of the Drug-Loaded Micro-Powders

#### 3.9.1. Particle Size and Dimensional Distribution

Particle size characterization of the microparticles was carried out by means of the sieving method. The total mass of microparticles recovered at the end of the drying process of each batch was weighed and placed on the uppermost of a stack of six standard stainless-steel sieves (Endecotts Ltd., London, UK) arranged in descending order with mesh apertures of 450, 300, 250, 180, 106, and 90  $\mu\text{m}$ . Using a mechanical sieve shaker (Endecotts, Octagon 200), the sieves were subjected to continuous vibration for 15 min. At the end of the process, the fraction retained on each sieve was collected and weighed. The results are expressed as the mean percentage size distribution ( $n = 5$ )  $\pm$  SE. For each batch, the fraction above 450  $\mu\text{m}$  was subsequently discarded.

#### 3.9.2. Morphology

The microparticle morphology was examined by optical microscopy using a Reichert-Jung Microstar 110 (Vienna, Austria) optical microscope (10 $\times$  and 4 $\times$  magnification). Morphological assessment was performed both before and after sieving in order to evaluate the morphological features upon variation in particle size.

### 3.10. Bulk Properties of the Drug-Loaded Micro-Powders

#### 3.10.1. Volumes and Densities

The bulk and tapped volumes were determined according to the Ph. Eur. 11th Ed. All batches prepared with the same composition were pooled, weighed, and transferred into a graduated cylinder. The volume occupied was recorded as the bulk volume ( $V_{\text{BULK}}$ ). Subsequently, a series of taps (10, 500, or 1250) was applied to the base of the cylinder to ensure appropriate packing of the microparticles and a reduction in interparticulate spaces. The tapping process was continued until no further change in powder volume was observed, and the tapped volume ( $V_{\text{TAPPED}}$ ) was subsequently calculated. Thus, the

obtained volume values were used to calculate the bulk density ( $\rho_{BULK}$ ) and tapped density ( $\rho_{TAPPED}$ ) as follows:

$$\rho_{BULK} = \frac{Weight\ (g)}{V_{BULK}\ (mL)} \quad \rho_{TAPPED} = \frac{Weight\ (g)}{V_{TAPPED}\ (mL)}$$

The experiments were conducted in triplicate, and the results are reported as means ( $n = 3$ )  $\pm$  SE.

### 3.10.2. Compressibility Index and Hausner Ratio

Using the previously determined values of bulk and tapped density, the Compressibility index (%) and Hausner ratio were calculated, according to Ph. Eur 11th Ed., as follows:

$$Compressibility\ index\ \% = \frac{\rho_{TAPPED} - \rho_{BULK}}{\rho_{TAPPED}} \times 100$$

$$Hausner\ ratio = \frac{\rho_{TAPPED}}{\rho_{BULK}}$$

The results are reported as the mean ( $n = 3$ )  $\pm$  SE.

### 3.10.3. Flowability

To evaluate the flow properties of the microparticles, the angle of repose was measured, as described in Ph. Eur 11th Ed. A stainless-steel instrument (Copley Scientific Flowability Tester, Model BEP2, Nottingham, UK) consisting of a funnel with an adjustable orifice was employed. Accurately weighed quantities of microparticles were allowed to flow through the funnel and fall onto a base with a radius of 2.75 cm ( $r$ ). The height of the resulting cone ( $h$ ) was measured using a dedicated device (Mitutoyo Absolute Digimatic Height Gauge, Kawasaki, Japan). The angle of repose ( $\Phi$ ) was then calculated as follows:

$$\tan \Phi = \frac{h}{r}$$

The experiments were repeated six times, and the results are reported as the mean ( $n = 6$ )  $\pm$  SE.

### 3.11. Preparation of the Microparticle-Loaded Buccal Patches

To obtain the microparticle-loaded buccal patches, MLC-RSV-F and/or SLM-ERY-25 were used. The patches loaded with only SLM-ERY-25 were indicated as ERY-patches, whereas the patches containing both microparticles were indicated as ERY + RSV-patches.

For the preparation of all the microparticle-loaded patches, a base gel was first obtained using distilled water (DW) or one of the citrate buffers previously described in terms of composition, pH, and molarity (CB1, CB2, CB3, or CB4) as a solvent. An accurately weighed amount (Ohaus SCOUT™ SKX123, New Jersey, USA) of aqueous phase was heated to  $60.0 \pm 0.5$  °C in a ground-glass stoppered flask, then the following components were solubilized/dispersed in sequence: potassium sorbate, trehalose, PVP K30, and HEC. Each component was only added after complete solubilization/dispersion of the preceding one. The mixture was kept under continuous stirring using a magnetic stirrer. The resulting transparent gel was placed in an ultrasonic bath (Branson 1200, Connecticut, USA) for approximately 1 h to remove entrapped air microbubbles and promote structural uniformity of the system. The gel was subsequently stored at 4 °C overnight to allow stabilization of the polymer network.

For patch preparation, a predetermined amount of the base gel was accurately weighed into a beaker, and predefined quantities of MLC-RSV-F and/or SLM-ERY-25 were incorporated. The weight ratios of all components are reported in Table 8.

**Table 8.** Qualitative–quantitative composition of the dispersions employed to produce the buccal patches.

|                          | ERY-Patches | ERY + RSV-Patches |
|--------------------------|-------------|-------------------|
| <i>Potassium sorbate</i> | 0.46 mg     | 0.46 mg           |
| <i>Trehalose</i>         | 24 mg       | 24 mg             |
| <b>Gel base (4.58 g)</b> | 24 mg       | 24 mg             |
| <i>PVP K30</i>           | 132 mg      | 132 mg            |
| <i>HEC</i>               | 4.39 g      | 4.39 g            |
| <i>Aqueous medium</i>    | 320 mg      | 320 mg            |
| <b>SLM-ERY-25</b>        | /           | 100 mg            |
| <b>MLC-RSV-F</b>         |             |                   |

The obtained suspensions were manually mixed using a glass rod, applying slow and continuous circular movements to ensure uniform distribution while minimizing air incorporation and preventing bubble formation. Once a homogeneous mixture was achieved, 4.90 g of the dispersion containing SLM-ERY-25 or 5.00 g of the suspension containing both SLM-ERY-25 and MLC-RSV-F were poured into a square silicone mold (surface area: 20.25 cm<sup>2</sup>) and left to dry in an oven at 30 °C for 48 h in the presence of CaCl<sub>2</sub> (RH: 70%). The resulting patches were equilibrated at room temperature for 2 h, accurately weighed, sealed in polyethylene sachets, and stored at 4 °C. The ERY+RSV-patches, owing to the presence of RSV, were additionally stored in the dark. A total of 10 different types of microparticle-loaded patches were prepared, varying the aqueous solvent used, as detailed in Table 9.

**Table 9.** Compositions and formula codes of the prepared buccal patches.

| Patch Formula Code                   | Aqueous Medium | GEL Code           | SLM-ERY-25 | MLC-RSV-F |
|--------------------------------------|----------------|--------------------|------------|-----------|
| <i>ERY-patch<sub>DW</sub></i>        | DW             | Gel <sub>DW</sub>  | ✓          | X         |
| <i>ERY + RSV-patch<sub>DW</sub></i>  | DW             | Gel <sub>DW</sub>  | ✓          | ✓         |
| <i>ERY-patch<sub>CB1</sub></i>       | CB1            | Gel <sub>CB1</sub> | ✓          | X         |
| <i>ERY + RSV-patch<sub>CB1</sub></i> | CB1            | Gel <sub>CB1</sub> | ✓          | ✓         |
| <i>ERY-patch<sub>CB2</sub></i>       | CB2            | Gel <sub>CB2</sub> | ✓          | X         |
| <i>ERY + RSV-patch<sub>CB2</sub></i> | CB2            | Gel <sub>CB2</sub> | ✓          | ✓         |
| <i>ERY-patch<sub>CB3</sub></i>       | CB3            | Gel <sub>CB3</sub> | ✓          | X         |
| <i>ERY + RSV-patch<sub>CB3</sub></i> | CB3            | Gel <sub>CB3</sub> | ✓          | ✓         |
| <i>ERY-patch<sub>CB4</sub></i>       | CB4            | Gel <sub>CB4</sub> | ✓          | X         |
| <i>ERY + RSV-patch<sub>CB4</sub></i> | CB4            | Gel <sub>CB4</sub> | ✓          | ✓         |

Each patch type was prepared in triplicate, except for ERY-patch<sub>CB2</sub> and ERY + RSV-patch<sub>CB2</sub>, which were prepared five times. The results are expressed as the mean ( $n = 3$  for all patches;  $n = 5$  for ERY-patch<sub>CB2</sub> and ERY + RSV-patch<sub>CB2</sub>)  $\pm$  SE.

For ERY-patch<sub>CB2</sub> and ERY + RSV-patch<sub>CB2</sub>, the residual water content % was determined as follows:

$$\text{Residual water content \%} = \frac{\text{Weight}_{\text{experimental}} - \text{Weight}_{\text{theoretical}}}{\text{Weight}_{\text{experimental}}} \times 100$$

where Weight<sub>experimental</sub> is the measured weight of the patch and Weight<sub>theoretical</sub> is the sum of dry components constituting one patch. The results are reported as the mean ( $n = 5$ )  $\pm$  SE.

### 3.12. pH Assessment of Preparation Intermediates and Patches

During all preparation steps leading to the production of the microparticle-loaded patches, pH measurements were carried out. Measurements were performed using a properly calibrated pH meter (pH meter HI 2211 pH/ORP Meter, Hanna Instrument (Woonsocket, RI, USA)). The appropriate amount of each sample was diluted/dispersed in water or in pH 6.8 artificial saliva, mixed with the aid of a vortex (LLG-uniTEXTER), and then analyzed. Specifically:

- 0.94 g of each base gel was diluted in 5 mL of water and immediately analyzed.
- 1 g of each microparticle-loaded gel was diluted in 5 mL of water and immediately analyzed.
- Dry patch discs with a surface area of  $0.66 \text{ cm}^2$  ( $\approx 20 \text{ mg}$ ) were immersed in 1 mL of water or artificial saliva at pH 6.8, and analyzed after 30 min of incubation to allow swelling of the polymer matrix.

Each measurement was performed in triplicate, and the results are reported as the mean ( $n = 3$ )  $\pm$  SE.

### 3.13. Folding Endurance

To evaluate the flexibility and folding resistance of the buccal patches, samples were repeatedly folded at the same point and at different positions of the same patch until structural failure occurred, or up to a maximum of 300 folds. The maximum number of folds sustained by each patch prior to rupture was recorded and reported as the folding endurance value [42].

Based on the results, further characterizations were performed only on the ERY-patch<sub>CB2</sub> and ERY + RSV-patch<sub>CB2</sub> formulations.

### 3.14. Uniformity Evaluation of ERY-patch<sub>CB2</sub> and ERY + RSV-patch<sub>CB2</sub>: Weight, Thickness, and Active(s) Content

To assess weight uniformity, 3 discs with surface areas of  $0.52 \text{ cm}^2$  were cut from each patch and accurately weighed on an analytical balance with sensitivity to the fifth decimal (METTLER AE 240). The results are expressed as the mean ( $n = 15$ )  $\pm$  SE.

The thickness of each patch was measured at 5 distinct points (the center and the four corners) using a digital micrometer (DIN 863, Vogel GmbH & Co. KG, Kevelaer, Germany) with a measurement range of 0–25 mm and a sensitivity of 0.001 mm. The results are reported as the mean ( $n = 25$ )  $\pm$  SE.

For the quantitative determination of ERY and RSV (when present), 2 discs with a surface area of  $0.196 \text{ cm}^2$  were cut from each patch, weighed, transferred into 20 mL volumetric flasks, and brought to volume with a water–methanol mixture (1:4, *v/v*). The dispersions were vigorously mixed using a vortex and then sonicated in an ultrasonic bath for 30 min. The resulting solutions were centrifuged, filtered, and subsequently analyzed by HPLC-DAD, as previously reported. To quantify RSV, a calibration curve was constructed, analyzing five methanolic standard solutions of RSV within a concentration range of 0.1–5.0  $\mu\text{g/mL}$ :  $\lambda_{\text{max}} = 305 \text{ nm}$ , retention time = 11.3 min, linear regression equation:  $\text{Area} = 140,915.24 \times C \text{ (mg/mL)}$  ( $R = 0.999$ ). The results are reported in terms of active(s) amount per unit area ( $\text{mg/cm}^2$ ) and DL%. The results are reported as the mean ( $n = 10$ )  $\pm$  SE.

### 3.15. Swelling Studies

To determine the swelling index of the ERY-patch<sub>CB2</sub> and ERY + RSV-patch<sub>CB2</sub> formulations, discs with a surface area of  $0.38 \text{ cm}^2$  were accurately weighed (initial weight,  $W_0$ ), placed on a watch glass, treated with 100  $\mu\text{L}$  of artificial saliva pH 6.8, and incubated at room temperature in the dark if containing RSV for predefined time intervals: 5, 15, 30,

45, 60, 75, and 90 min. At each time point, the samples were blotted with filter paper to remove excess liquid and subsequently weighed (weight at time  $t$ ,  $W_t$ ) in order to calculate the Swelling index % as follows [32]:

$$\text{Swelling index \%} = \frac{W_t - W_0}{W_0} \times 100$$

The experiment was performed in duplicate, and the results are reported as the mean ( $n = 2$ )  $\pm$  SE.

Furthermore, the dimensional increase in the patches due to the swelling phenomenon was evaluated in terms of both diameter and thickness changes. For this purpose, discs with a surface area of  $0.38 \text{ cm}^2$  were placed on a microscope slide over a sheet of graph paper. Samples were wetted with  $100 \mu\text{L}$  of artificial saliva at pH 6.8 and observed for up to 90 min. During the experiment, images were captured to monitor dimensional changes in the planar and frontal directions. These data were used to calculate the patch diameter and thickness as a function of time. The experiments were performed in duplicate, and the results are reported as the mean ( $n = 2$ )  $\pm$  SE.

### 3.16. Mucoadhesion

#### 3.16.1. Buccal Porcine Tissue Preparation

Buccal mucosa samples were obtained from the vestibular region of the retromolar trigone of approximately 12-month-old domestic pigs intended for human consumption. Immediately after sacrifice, the samples were transferred into refrigerated containers and transported to the laboratories within 3 h of collection. Excess connective and adipose tissue were removed, and the samples were washed with an isotonic solution. Subsequently, the tissues were immersed for 60 min in isotonic solution containing trehalose (5%  $w/v$ ) as a cryoprotectant, then drained, blotted, and stored in sealed polyethylene bags at  $-80 \pm 1^\circ\text{C}$  (Thermo Forma Ultra-Freezer  $-86^\circ\text{C}$ , model 902, Thermo Scientific, Waltham, MA, USA) until further use [43].

#### 3.16.2. Qualitative Evaluation

The previously described porcine tissue samples were thawed and conditioned in artificial saliva at pH 6.8 at  $37.0 \pm 0.5^\circ\text{C}$  for 10 min. Tissue samples were then placed on Petri dishes, and a section of ERY-patch<sub>CB2</sub> and ERY + RSV-patch<sub>CB2</sub> ( $2 \times 1 \text{ cm}$ ) was applied to the mucosal surface. To qualitatively assess the adhesive properties of the patches, each tissue sample was inverted so that the adhered patch was subjected to gravitational force, and any detachment was observed. Furthermore, to evaluate the patch's ability to remain attached at the application site under normal oral cavity movements, the samples were subjected to repeated mechanical stress. The experiment was performed in triplicate for each formulation, and photographs and videos were recorded during each experiment.

#### 3.16.3. Quantitative Evaluation

Prior to performing the mucoadhesion evaluation, the porcine mucosal epithelium was separated from the underlying connective tissue. After at least two weeks of storage at  $-80^\circ\text{C}$ , the still-frozen tissues were subjected to thermal shock by immersion in a preheated isotonic solution at  $60.0 \pm 0.5^\circ\text{C}$  for 1 min in order to manually separate the mucosal layer from the underlying structures using tweezers [43]. The mucoadhesive properties of the patches were evaluated using a Texture Analyzer TX-700 (Lamy Rheology, Champagne au Mont d'Or, France), equipped with a 10 N load cell and a cylindrical probe (Model TX-BLMPEG; diameter 12.7 mm, height 30 mm; contact area:  $1.26 \text{ cm}^2$ ) made of polymethylmethacrylate (PMMA).

The porcine buccal mucosa was secured on a custom-made PMMA support and kept moist with 50 µL of artificial saliva at pH 6.8 or 50 µL of artificial saliva at pH 6.8 containing mucin (1% *w/v*). Simultaneously, patches discs (area 1.26 cm<sup>2</sup>) were applied to the lower surface of the probe using a double-sided adhesive tape. The quantitative mucoadhesion study was conducted as follows:

1. At rest, the mucosa and the patch were placed with an interspace of 1 mm, with no force applied by the probe on the tissue.
2. The experiment was initiated at the onset of probe descent, allowing firm interaction between the mucosa and the formulation (probe descent rate: 0.2 mm/s; compression distance: 1 mm).
3. The probe applied a constant force for a predetermined time (10, 15, 20, 25, 30, 35, 45, or 60 s; hold position: 1 mm).
4. The probe returned to the rest position (ascension rate: 0.1 mm/s), measuring the force required to detach the formulation from the mucosal surface (detection threshold: 0.04 N).

Data were extracted as applied force (N) as a function of time (s) or applied force (N) as a function of distance (mm). The adhesive force was then normalized to the contact area, obtaining the detachment force as follows:

$$\text{Detachment force (N/m}^2\text{)} = \frac{\text{Force of adhesion (N)}}{\text{Contact surface (m}^2\text{)}}$$

The experiments were repeated six times, and the results are reported as the mean ( $n = 6$ )  $\pm$  SE.

### 3.17. Ex Vivo Studies

#### 3.17.1. Ex Vivo Permeation Studies

Prior to performing the ex vivo experiment, the buccal mucosa was conditioned and washed overnight in an isotonic solution. It was subsequently equilibrated at room temperature and repeatedly rinsed with a fresh isotonic solution to remove any residual biological material that might interfere with subsequent quantitative analyses. Appropriate portions of the mucosa were then excised and positioned between the donor and acceptor compartments of a Franz-type vertical diffusion cell (Permeagear, amber, unjacketed, flat flange joint, 0.636 cm<sup>2</sup> orifice, 15 mL acceptor volume; SES GmbH-Analysesysteme, Bechenheim, Germany) filled with CB1 and CB1 containing 3% (*w/v*)  $\beta$ -cyclodextrins, respectively.

The cells were equilibrated for approximately 15 min at  $37.0 \pm 0.5$  °C. The donor compartment solution was then removed and immediately replaced with a patch disc (area 0.52 cm<sup>2</sup>) soaked with 0.2 mL of CB1. The system was maintained at  $37.0 \pm 0.5$  °C and, for the RSV-containing patch, protected from light for variable durations up to a maximum of 2 h. At predetermined time points, 0.4 mL aliquots were withdrawn from the acceptor compartment and immediately replaced with an equal volume of fresh acceptor fluid to maintain sink conditions. The collected samples were immediately frozen and subsequently subjected to freeze-drying (Labconco FreeZone 2.5, Kansas City, MO, USA) for 24 h. The resulting residues were re-dispersed in 400 µL of methanol and centrifuged, and the supernatant was filtered (0.45 µm pore diameter) and analyzed using HPLC-DAD as previously described. Each experiment was performed 5 times, and the results are reported as the mean ( $n = 5$ )  $\pm$  SE.

#### 3.17.2. Evaluation of the Active(s) Amount Entrapped into the Buccal Tissue

At the end of the permeation studies, the Franz cells were disassembled, and the mucosal membranes were rinsed with distilled water to remove any residual material

adhering to the mucosal surface. Subsequently, the mucosa was subjected to hot extraction by immersion in 2 mL of methanol preheated to  $60.0 \pm 0.5$  °C for 2 min. This procedure was repeated twice. The extraction liquors were collected in a 5 mL amber volumetric flask and brought to volume with methanol. Quantification of the extracted active compound(s) was carried out by HPLC-DAD analysis as previously described. Each experiment was performed 5 times, and the results are expressed as the mean ( $n = 5$ )  $\pm$  SE.

### 3.18. Antibacterial Activity Assay

The ability of ERY-patch<sub>CB2</sub> and ERY + RSV-patch<sub>CB2</sub> to inhibit the growth of surface-adherent bacterial cells was evaluated against the Gram-positive *Staphylococcus aureus* ATCC 25923 and the Gram-negative *Escherichia coli* ATCC 25922 using an agar diffusion assay, as previously described [44]. Briefly, a single colony of each strain was pre-cultured in tryptic soy broth (TSB) at 37 °C for approximately 16 h under shaking conditions (180 rpm). TSB consisted of 17 g/L casein peptone, 3 g/L soya peptone, 5 g/L sodium chloride, 2.5 g/L dipotassium hydrogen phosphate, and 2.5 g/L glucose, and was solidified with 15 g/L bacteriological agar when required. Bacterial suspensions were adjusted to approximately  $10^8$  colony-forming units per milliliter and evenly spread onto TSB agar plates to obtain confluent growth. ERY-patch<sub>CB2</sub> and ERY + RSV-patch<sub>CB2</sub>, as well as an empty control patch (i.e., hydrophilic matrix containing empty microparticles), were then placed onto the inoculated agar surface, and plates were incubated statically at 37 °C for 24 h. The experiment was performed in triplicate.

### 3.19. Data Analysis

The data are expressed as means  $\pm$  standard error (SE). All differences were statistically evaluated with Student's t-test or the one-way analysis of variance (ANOVA or F-test). Data were considered statistically significant when  $p < 0.05$ .

## 4. Conclusions

This study involved the development of erythromycin-loaded solid lipid microparticles (SLM-ERY) and resveratrol-loaded microstructured lipid carriers (MLC-RSV) as innovative, high-quality pharmaceutical powders for the formulation and characterization of mucoadhesive buccal patches. First of all, SLM-ERY and MLC-RSV were designed and characterized as lipid microparticles suitable for oromucosal administration. Using a “green” preparation method and optimized lipid composition, reproducible pharmaceutical powders with high loading efficiency ( $\approx 90\%$ ), spherical morphology, appropriate particle sizes, and a softening temperature close to body temperature were obtained. Then, the study involved the formulation and characterization of mucoadhesive buccal delivery systems specifically tailored to oromucosal loco-regional treatment, and potentially for the post-surgical therapy of patients at risk of or diagnosed with MRONJ. The buccal patches were produced by embedding SLM-ERY alone or in combination with MLC-RSV into a hydrophilic and deformable polymeric matrix. The patches, prepared via the solvent-casting technique, were found to be thin, flexible, and homogeneous in terms of thickness, weight, and active(s) content, and thus easily customizable. Upon application, the patches interact with saliva, swell, and promote microparticle release without any significant increases in thickness, thereby preventing patient discomfort. Moreover, the developed formulations exhibited strong mucoadhesive properties occurring after short contact times, ensuring effective adhesion to the oral mucosa, patient compliance, and prolonged residence time in situ. Furthermore, this allowed sustained release via softening of the microparticles upon reaching body temperature and diffusion of the active(s) into the underlying tissue due to the partition of the lipid excipients of the microparticles into the mucosa owing to their

chemical compatibility with the tissue components. Moreover, the intrinsic flexibility of the patches enables them to adapt to the physiological movements of the oral mucosa while maintaining integrity and adhesion for at least two hours, during which time-dependent ERY and RSV buccal accumulation was observed. In conclusion, two promising candidates for future clinical studies were proposed, aimed at evaluating the therapeutic efficacy of the co-administration of RSV and ERY in MRONJ treatment. This innovative approach shows potential to enhance therapeutic outcomes compared with the currently available strategies, offering more effective and patient-friendly pharmacological management of the target disease while also representing a valuable strategy for the topical oromucosal administration of antibiotics, which may be applicable in other therapeutic contexts as well (e.g., prevention of bacterial infections following tooth extraction), as evidenced by the proven antibiotic activity of the formulations. Certainly, deeper evaluations should be performed in the future in order to assess the frequency of administration and better understand how to customize the treatment in terms of patch size and, consequently, the active(s) amount. Indeed, the literature reports a large range of MIC values for ERY when varying the tested microorganism, e.g., 2–16 µg/mL against *Streptococcus dysgalactiae* [45], 0.25–2048 µg/mL for *Staphylococcus aureus*, 0.125–2048 µg/mL for *Staphylococcus epidermidis* [46], 0.06–0.12 µg/mL for *Bordetella pertussis* [47], etc. Considering the results from the ex vivo studies, the application of a very small 0.38 cm<sup>2</sup> patch disk could deliver 0.06 to 0.18 mg of ERY into the mucosal tissue by varying the formulation (ERY + RSV-patch<sub>CB2</sub> > ERY-patch<sub>CB2</sub>) and/or the considered time point. Again, concerning RSV, the literature reports a wide concentration range for each of its actions. As an example, in terms of osteoinduction, concentrations in the range of 1–25 µM show positive effects on osteoblastic activity and differentiation in vitro, with optimal effects often between 5 and 10 µM, yet it depends on the cell model used (e.g., Human Bone Marrow-Derived MSCs, ST2 mesenchymal cells, Rat and Human ADSCs, Osteoblasts) [12]. Despite the low RSV content in the ERY + RSV-patch<sub>CB2</sub>, as well as the low RSV retention in the mucosal tissue (≈2% of the administered dose), the application of a 0.38 cm<sup>2</sup> patch disk could deliver ≈1.2 µg of RSV into the mucosa. These speculative considerations offer a rationale that strongly supports further consideration and testing of the formulations presented here.

## 5. Patents

The work reported in this manuscript is covered by Italian patent application No. IT 201900011436, filed by Viviana De Caro and Libero Italo Giannola.

**Supplementary Materials:** The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/antibiotics15020151/s1>, Table S1. Correlation between compressibility index (%), Hausner ratio, and flow properties (Ph. Eur.); Figure S1. Pictures of the buccal patches; Figure S2. Pictures from the swelling/disintegration assay: ERY-patch<sub>CB2</sub>; Figure S3. Pictures from the swelling/disintegration assay: ERY + RSV-patch<sub>CB2</sub>; Figure S4. Ex vivo qualitative mucoadhesion test: ERY-patch<sub>CB2</sub> adhesiveness (as an example) vs. gravitational force.

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