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Mucoadhesion and Mucopenetration of Cannabidiol (CBD)-Loaded Mesoporous Carrier Systems for Buccal Drug Delivery

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Abstract: Transmucosal drug delivery represents a promising noninvasive option when drugs are employed which have a low oral bioavailability like CBD. However, this concept can only be successful as long as the formulation provides sufficient buccal retention and mucosal penetration. In this study, mucoadhesive carrier systems were evaluated consisting of CBD-loaded silica (Aeroperl 300) carriers, mucoadhesive polymers (Hypromellose (HPMC), chitosan and carbomer) and propylene glycol as a penetration enhancer. Mucoadhesive effect, drug release and penetration ability were evaluated for each carrier system. The results show that the addition of HPMC and carbomer substantially improve mucoadhesion compared to pure CBD, with an increase of 16-fold and 20-fold, respectively. However, due to their strong swelling, HPMC and carbomer hinder the penetration of CBD and rely on co-administration of propylene glycol as an enhancer to achieve sufficient mucosal absorption. Chitosan, on the other hand, achieves an 8-fold increase in mucoadhesion and enhances the amount of CBD absorbed by three times compared to pure CBD. Thus, chitosan represents a promising polymer to combine both effects. Considering the results, the development of silica-based buccal drug delivery systems is a promising approach for the effective delivery of CBD.

Keywords: buccal drug delivery; mucoadhesion testing; mucopenetration; mesoporous silica; incipient wetness method; cannabidiol release

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1. Introduction

Cannabidiol (CBD) is a non-psychoactive cannabinoid isolated from the *Cannabis sativa* plant [1] or derived by chemical synthesis [2]. It has attracted extensive and growing scientific and commercial interest due to its range of potential beneficial effects, including effects on anxiety, memory, locomotion, inflammatory reactions and pain perception [3]. Despite the variety of possible indications, only a few licensed medicinal products are presently available, including one approved pure CBD-based product for the treatment of a rare pediatric form of epilepsy [4]. In terms of drug delivery, the per oral administration of CBD proves to be particularly challenging, as CBD with both a high lipophilicity and a pronounced first-pass effect exhibits low bioavailability and variable pharmacokinetic profiles [5]. The bioavailability of CBD after per oral administration is estimated at 6% [6] and is highly susceptible to food effects [7]. Considering these challenging characteristics of CBD, there is a profound need for alternative routes of administration to enable successful therapy. Given its ease of administration and high patient acceptance, buccal administration represents an attractive and feasible non-invasive route of delivery [8]. The buccal mucosa, with its rich blood supply, provides direct access to the systemic circulation [9] and thus the potential to increase bioavailability by avoiding the hepatic first-pass effect and preventing degradation in the gastrointestinal tract [8]. Limitations in the buccal route may occur due to the small absorption area and to inadequate permeability of the mucosa [10]. Therefore, the development of buccal dosage forms often requires the employment of

suitable penetration enhancers. Ideally, the enhancer used should be safe, non-irritating and its effect should be reversible so that the integrity and barrier properties of the tissue are quickly restored [11]. Therefore, chitosan, as a biocompatible and non-toxic polymer, together with its penetration enhancing properties, is a desirable option for penetration enhancement in the oral cavity [12]. Another promising option in the search for a substance classified as safe with penetration-enhancing properties is propylene glycol; which is already widely used in dermal formulations as a co-solvent and/or to enhance drug penetration [13]. Beyond penetration, buccal drug delivery systems must maintain intimate contact with the mucosa long enough to allow drug release and absorption [8] and prevent drug loss by saliva flow in the oral cavity [14]. To counteract and prevent insufficient residence time of the drug on the mucosa, mucoadhesive polymers are incorporated in buccal delivery systems. However, there is no approved polymer-based CBD delivery system available yet. Sativex, an approved CBD-based oromucosal spray, does not contain any mucoadhesive polymer. Hence, recent studies for the oromucosal spray have implied that a substantial portion of the administered dose is washed from the mucosa and may subsequently be absorbed through the gastrointestinal tract [15,16].

Mesoporous silica are generally considered to be excellent carriers for drug delivery systems owing to their unique properties, including biocompatibility, drug loading capability and subsequent controlled release at the target site. In addition, silica materials are commonly used as excipients in numerous oral products and are therefore attractive carriers for buccal drug delivery systems [17,18].

In this study, mucoadhesive carrier systems for the buccal transmucosal delivery of CBD were investigated, consisting of a mesoporous silica as a carrier for CBD and a mucoadhesive polymer for the prolonged contact time. Three promising polymer candidates were investigated, namely nonionic hypromellose (Metolose 65SH50), anionic acrylic acid polymer (Carbopol 971P NF) and cationic chitosan. The mucoadhesive properties of the carrier systems and their susceptibility to salivary flow were determined using an in vitro test model. Furthermore, drug release and the mucosal penetration behavior of CBD from the mucoadhesive formulations were evaluated. Propylene glycol was incorporated into the carrier systems as an enhancer to provide promotion of mucosal absorption. Mucoadhesion and mucopenetration were reassessed to investigate the effect of propylene glycol on the adhesion and penetration behavior of CBD.

2. Materials and Methods

2.1. Materials

Synthetically produced Cannabidiol (Canapure > 98% CBD) was kindly provided by Symrise AG (Holzminden, Symrise AG, Holzminden, Germany). Metolose 65SH50 was donated by Shin-Etsu Chemical Co. (Tokyo, Japan), Carbopol 971P NF from Lubrizol Advanced Materials Europe BVBA (Brussels, Belgium), Aeroperl 300 Pharma from Evonik Resource Efficiency GmbH (Hanau, Germany) and Kolliphor PS 80 Ph.Eur. by BASF SE (Ludwigshafen, Germany). Food-grade chitosan was purchased from Harke Pharma GmbH (Mülheim-Ruhr, Germany) and acetonitrile (HPLC grade) from J.T. Baker, Avantor performance Materials B.V. (Deventer, The Netherlands). Hydrochloric acid PF 37%, ethanol (HPLC grade), α–Amylase from *Bacillus subtilis* (380 U/mg) and mucin from porcine stomach type III were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Potassium chloride from VWR International GmbH (Leuven, Belgium) and sodium chloride from Caesar & Loretz GmbH (Hilden, Germany) were Ph.Eur. grade. Refined sunflower oil was purchased from Caesar & Loretz GmbH (Hilden, Germany). Dipotassium hydrogen phosphate trihydrate (Ph.Eur. grade) was purchased from Merck KGaA (Darmstadt, Germany), NEG 50 from Thermo Fisher Scientific Inc. (Waltham, MA, USA) and aluminum foil from FORA GmbH (Radolfzell, Germany). Propylene glycol was donated from Dr. Willmar Schwabe GmbH & Co. KG (Karlsruhe, Germany), Parafilm M from Bemis Company, Inc. (Oshkosh, WI, USA) and Chromafil Xtra H-PTFE-20/25 from Macherey-Nagel GmbH & Co. KG (Düren, Germany). Porcine buccal mucosa was supplied

by the Department of Experimental Medicine at the University of Tuebingen and a local butcher. The Department of Pharmaceutical Technology is registered for the use of animal products at the District Office of Tübingen (registration number: DE 08 416 1052 21).

2.2. Preparation of CBD-Loaded Carrier Systems

Prior to the loading process, the mesoporous silica (Aeroperl 300) was dried for 30 min at 110 °C. Loading of Aeroperl 300 with CBD was carried out in a LabMixer (Somakon Verfahrenstechnik UG, Lünen, Germany). To this end, 15 g of silica carrier were placed in the mixer and loading was done by steadily syringing an ethanolic CBD solution (3 g CBD in 3 mL ethanol $\geq$ 99.8% (*v/v*)) to the carrier whilst mixing at low speed for 4 min. Finally, the loaded carrier was mixed for another 10 min at 600 rpm with the scraper (level 2) turned on. Subsequently, the silica carrier was dried at room temperature until the ethanol was evaporated and mass constancy was reached.

2.2.1. Preparation of CBD-Loaded Carrier Systems with Propylene Glycol

CBD-loaded carrier systems with propylene glycol were obtained by adding propylene glycol to the ethanolic CBD solution. The concentration of propylene glycol in the solution was chosen depending on the desired final concentration of propylene glycol in the carrier system. Loading of the Aeroperl 300 was accomplished as described above (2.2 Preparation of CBD-loaded carrier systems).

2.3. Preparation of Mucoadhesive Carrier Systems

Mucoadhesive carrier systems were prepared by loading the mesoporous silica with CBD (2.2 Preparation of CBD-loaded carrier systems) and afterwards coating it with a mucoadhesive polymer in a LabMixer. A suspension consisting of either carbomer, HPMC or chitosan in sunflower oil was prepared. The final concentration of polymer in the carrier system was controlled by the amount of suspension and its concentration. Subsequently, the suspension was dosed dropwise to 15 g of CBD-loaded carrier. While adding the polymer suspension, the rotational speed was first adjusted to 400 rpm for 5 min and then raised to 600 rpm for 2 min. Finally, the scraper was set to level 2 for 5 min.

Preparation of Mucoadhesive Carrier Systems with Propylene Glycol

For the preparation of mucoadhesive carrier systems with propylene glycol, 15 g of CBD-loaded carrier system with propylene glycol were prepared according to Section 2.2.1 (Preparation of CBD-loaded carrier systems with propylene glycol). The subsequent coating was performed in accordance with the method described above (2.3 Preparation of mucoadhesive carrier systems).

2.4. Drug Load Quantification

To determine the amount of CBD loaded into the silica carriers, three sample aliquots of about 10 mg each were extracted with 1 mL of ethanol for at least 1 h. After centrifuging for 5 min at 13,400 rpm, the supernatant was analyzed by high performance liquid chromatography (HPLC). The CBD load is expressed in % (m/m).

2.5. High Performance Liquid Chromatography Assay

Quantitative analysis of the CBD concentration in the samples was performed with a HPLC system (Shimadzu LC-20AT Prominence, DGU-20A5R degasser, SIL-20AC H autosampler, CTO-10ASVP oven, CBM-20A communication bus module; Shimadzu GmbH, Duisburg, Germany) equipped with a UV-detector (SPD-20A; Shimadzu, Duisburg). The separation was conducted on a RP-18 column (Nucleosil 100-5 C18 125/4; Macherey-Nagel GmbH & Co. KG, Düren) at 30 °C. The mobile phase during determination was adjusted to a flow rate of 1 mL/min and was composed of acetonitrile:water (55:45 *v*/*v*). For analysis, sample volumes of 20 µL were injected.

2.6. Dissolution Experiments

CBD release from the prepared carrier systems was assessed using a Ph.Eur. paddle dissolution apparatus (Pharma Test PT-DT, Pharma Test Apparatebau AG, Hainburg, Germany). Dissolution tests were carried out at 37 °C and with a stirring speed of 65 rpm. For the dissolution medium, 100 mL of artificial saliva supplemented with 0.5% polysorbate 80 to ensure sink-conditions was used. The composition of the artificial saliva was adapted from Hoffmann et al. [17]. Aliquots of 2 mL were collected after 2.5, 5, 10, 15, 30, 45, 60, 90, and 120 min. After filtration through a 0.20 µm hydrophilic PTFE filter, 300 µL of the aliquots were blended with 300 µL cold acetonitrile to precipitate the proteins. For further processing, the samples were vortexed for 10 s and then centrifuged at 13,400 rpm for 5 min. The supernatants were analyzed by HPLC (2.6 High performance liquid chromatography assay). Results were obtained from 3 replicates. The extracted loading amounts were used as the basis for calculating the percentage release in the dissolution experiments.

2.7. Mucoadhesion Test

Mucoadhesion of the prepared carrier systems was examined according to Hoffmann et al. [19]. In a slightly modified mucoadhesion cell (Figure 1), porcine buccal mucosa was fixed on a holder and the mucosa was warmed up to 36.5 °C ± 1 °C. Wetting the surface of the mucosa and the holder with 1 mL of artificial saliva prevented drying out during temperature regulation. Simultaneously, 10 mL of artificial saliva were added to the mucoadhesion cell to allow subsequent sampling. Approximately 20 mg of the samples were placed on the buccal mucosa and flushed with artificial saliva with a flow rate of 0.5 mL/min. The test was stopped after 12, 30 or 60 min, respectively. The remaining carrier was removed from the mucosa with a swab and 1 mL ethanol. The swab was extracted with 5 mL ethanol. The collected saliva was withdrawn from the mucoadhesion cell. Afterwards, the mucoadhesion cell was rinsed with 16 mL ethanol (respectively 25 mL and 40 mL for 30 min and 60 min testing time), which was then combined with the saliva to quantify the amount of CBD flushed from the mucosa. All samples were filtered with 0.20 µm hydrophilic PTFE filters. The saliva samples were then mixed with cold acetonitrile in equal parts, vortexed for 10 s and centrifuged at 13,400 rpm for 5 min, to precipitate the proteins. Assay was performed by HPLC. The mucoadhesion value represents the amount of retained CBD expressed as percentage of the total initial amount in the carrier system. In order to allow comparison, a mucoadhesion coefficient was calculated by dividing the mucoadhesion value of the carrier system by the mucoadhesion value of pure CBD. Each sample was tested in triplicate.

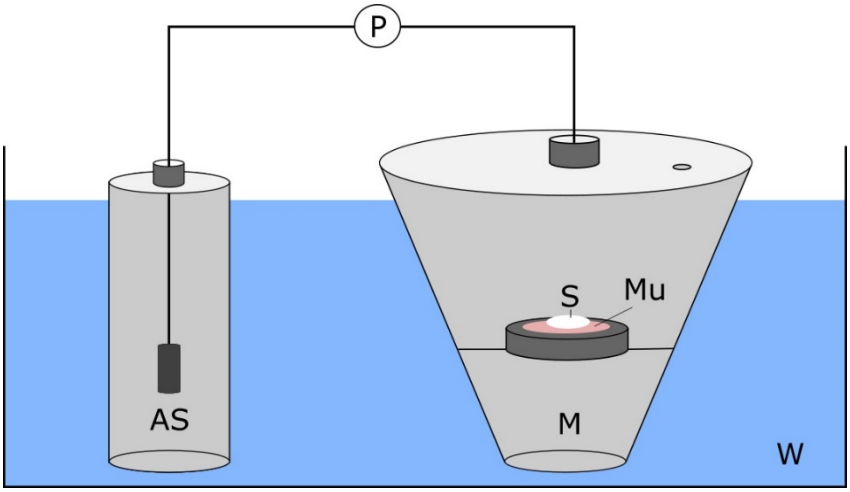


Figure 1. Mucoadhesion test system; AS: artificial saliva, P: pump, S: sample, Mu: mucosa, M: mucoadhesion cell, W: water bath.

2.8. Mucopenetration Studies

To assess penetration of CBD in the mucosa, the latter was removed from the mucoadhesion cell, then weighed and immediately frozen in liquid nitrogen. The frozen mucosa was placed in a container made of aluminum foil with the mucosal surface facing down. Afterwards, the lower surface was covered with a smooth layer of frozen sectioning medium (NEG 50). The object was flash frozen at −50 °C to solidify the medium. With a few more drops of NEG 50, the mucosa was attached to the sample holder of the cryomicrotome (HM 560 Cryo-Star; Thermo Fisher Scientific, Inc., US MA Waltham). The object temperature was set to −30 °C and the knife temperature to −32 °C to obtain an optimal longitudinal slice and to prevent thawing of the mucosa. The section thickness was set to 100 µm. The mucosa was then segmented slice by slice. The first slice, with a thickness of 100 µm, was taken as the first sample. Thereafter, five slices were prepared and reunited as the second sample. This process of collecting and pooling five slices was repeated until the complete mucosa was sectioned. For extraction of the drug from the mucosa, 1 mL of acetonitrile was added to each sample. After vortexing the samples for 5 s, they were subjected to the ultrasonic bath for 30 min. Afterwards, the samples were filtered (0.20 µm hydrophilic PTFE) and analyzed by HPLC.

Calculation of the amount penetrated per area was performed by normalizing the amount of CBD obtained to the quantity of CBD used, the weight of the mucosa and the area of mucosa exposed to the sample. To this end, an average mucosal weight of 1200 mg, an average applied amount of 3 mg of CDB and a penetration area of 2.08 cm^2 were assumed. Penetration experiments were carried out in triplicate. To simplify comparison, a penetration enhancement ratio (PE) was calculated by dividing the penetrated amount of CBD from the carrier system by the penetrated amount of pure CBD.

$$\mathrm{PE} = \frac{\text{Penetrated CBD } \left[\frac{\text{ug}}{\text{cm}^2}\right] \text{of carrier system}}{\text{Penetrated CBD } \left[\frac{\text{ug}}{\text{cm}^2}\right] \text{ of pure CBD}} \quad (1)$$

2.9. Microscopy

Images of physical mixtures of propylene glycol and CBD with ratios of 1:1, 1:2, 1:3 and 1:4 were taken with a light microscope. For this purpose, the samples were spread thinly on microscope slides and covered with a cover slip. A magnification of 20- or 40-fold was used to capture the microscopic images of the samples.

2.10. Differential Scanning Calorimetry

In order to investigate the physical state of CBD, samples were analyzed by means of differential scanning calorimetry. In addition to pure CBD, serving as a reference, CBD-loaded carrier systems and physical mixtures of propylene glycol and CBD in the ratios 1:1, 1:2, 1:3 and 1:4 were investigated. The samples were weighed in 40 µL aluminum crucibles and sealed. The scans were performed at a heating rate of 20 K/min under N_2 gas purging with a flow rate of 80 mL/min. For measuring the melting peak of CBD at 66−67 °C, the samples were heated from −30 °C to 90 °C. The thermal events were recorded using the STARe Evaluation Software.

2.11. Statisitcal Data Analysis

Whenever it was possible, results were presented as mean value of observed parameter ± SD. Statistical analysis was conducted with the help of GraphPad Prism 8.0 (GraphPad Software Inc., La Jolla, CA, USA). Statistical differences among multiple groups were evaluated though one-way ANOVA followed by Dunnett's post hoc test. Assessment of statistical significance in the values penetrated CBD from carrier systems with and without propylene glycol was performed through Student's *t*-test. Significant differences were marked with number of asterisks: * $p < 0.05$.

3. Results and Discussion

3.1. CBD-Loaded Carrier Systems

Drug loading into mesoporous silica (Aeroperl 300) was accomplished by the incipient wetness method. The applied volume of the ethanolic solution was approximately 11% of the pore volume and the achieved carrier loading was 16.48% ± 0.82% m/m related to the total mass. The loading process limits the formation of crystalline material by the confined space of the pores, leaving the incorporated drug in its non-crystalline, amorphous form [20]. No melting peak of crystalline CBD was obtained in the DSC measurement of the CBD-loaded mesoporous silica (Figure 2), indicating that CBD was fully incorporated into the silica carrier.

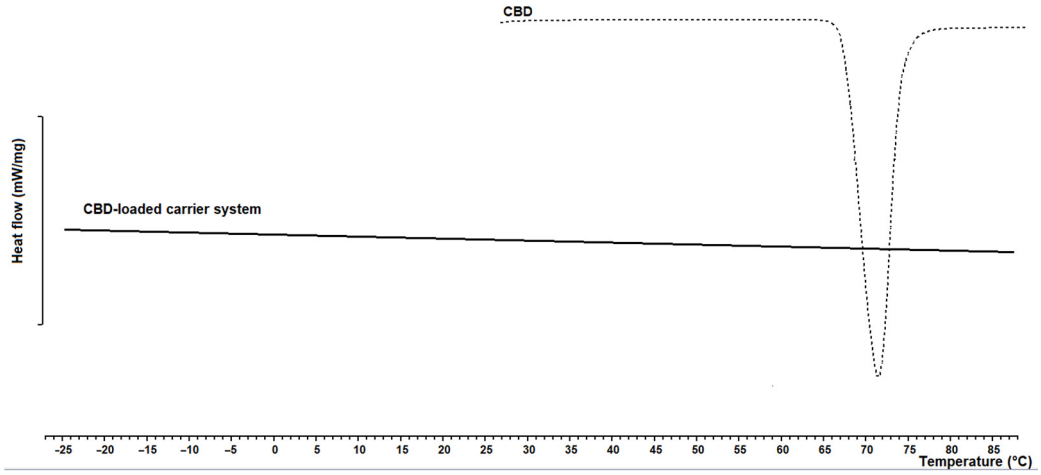


Figure 2. Comparison of DSC curves obtained by pure CBD and CBD loaded into Aeroperl 300.

3.2. CBD-Loaded Mucoadhesive Carrier Systems

3.2.1. Mucoadhesion Test of CBD-Loaded Mucoadhesive Carrier Systems

To improve the residence time of CBD at the absorption site, mucoadhesive polymers were added to CBD-loaded silica carriers. The polymers were introduced via a sunflower oil suspension. In order to achieve a high mucoadhesive effect, the introduction process aimed for the highest possible concentration of polymer. The maximum amount of polymer which can be added to the carrier system is limited by two aspects: the viscosity of the suspension, as well as the maximum oil binding capacity of the carrier, restricting the total amount of suspension which can be added. Considering these two factors, the maximum amount of polymer related to the total mass was 10% HPMC and 10% carbomer. In the case of chitosan, the limit was 6%. The mucoadhesion potential of the carrier systems with the aforementioned concentrations of polymers were determined using a mucoadhesion test model and compared with those of pure CBD and CBD incorporated in Aeroperl 300 as references. The results are shown in Table 1. Incorporation of CBD into Aeroperl 300, even without the addition of a mucoadhesive polymer, already resulted in an increase in the adherent fraction of CBD by more than 5-fold. Among all the carrier systems studied, the highest mucoadhesion value with about 80% CBD remaining on the mucosa was observed for carbomer, followed by HPMC with about 70% mucoadhesion and chitosan with approximately 30%. Overall, all carrier systems with polymer had a higher mucoadhesive effect compared to free and silica-bound CBD.

In order to study the mucoadhesion kinetic, the test was extended to 60 min. The results (Figure 3) revealed that carbomer yielded both high and long-lasting mucoadhesive effects. After 60 min, approximately 70% of the applied dose of CBD remained still on the mucosa. The mucoadhesive properties of the carrier systems with HPMC dropped more rapidly compared to those with carbomer, but it nevertheless showed a high mucoadhesion value of 45% after 30 min and a moderate value of 33% after 60 min. Despite the fact that the mucoadhesive properties of the carrier system with chitosan were less pronounced than

those of the carrier systems with the other two mucoadhesive polymers, the mucoadhesive effect was stable over a period of 60 min. After 60 min, about 20% CBD remained on the mucosa with the carrier system containing chitosan compared to approximately 10% with the carrier system without a mucoadhesive additive. The latter, exhibiting a mucoadhesion of approximately 10% after 60 min, still showed a superior mucoadhesive effect compared to pure CBD, which was almost completely flushed from the mucosa after 30 min.

Table 1. Mucoadhesion and mucoadhesion coefficient after 12 min mucoadhesion test (Plain carrier system: CBD-loaded polymer-free carrier system); n = 3; mean ± SD.

Formulation	Mucoadhesion [%]	Mucoadhesion Coefficient
CBD	3.94 ± 0.85	1.00
Plain carrier system	22.31 ± 1.98	5.66
Sunflower oil chitosan	32.82 ± 7.09	8.33
Sunflower oil HPMC	62.87 ± 3.76	15.96
Sunflower oil carbomer	77.65 ± 1.10	19.71

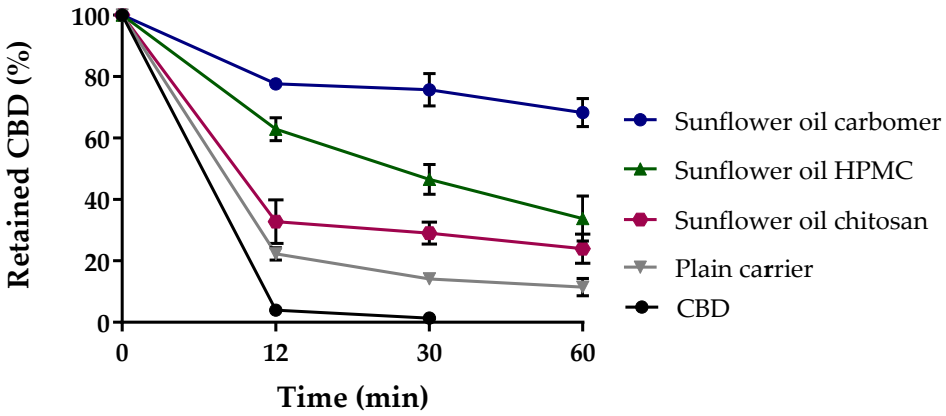


Figure 3. Mucoadhesion kinetic of CBD, CBD incorporated into Aeroperl 300 (Plain carrier) and mucoadhesive carrier systems with carbomer, HPMC and chitosan; n = 3; mean ± SD.

Carbomer is ranked among the strongest mucoadhesive polymers, while HPMC is known as a moderately mucoadhesive polymer [21,22]. The comparatively weaker retention time of HPMC can be attributed to the absence of carboxyl groups, which serve as proton donors for hydrogen bonds with the mucins [22]. The mucoadhesive properties of chitosan are reported to range from weak and short-lasting [23] to strong [24]. When comparing its effect in this study with carbomer and HPMC, it has to be considered that the applied concentration of chitosan was limited to 6% compared to 10% of the two others. The overall obtained mucoadhesion values are in good agreement with the reported properties of the used polymers in the literature. Due to its intrinsic properties and the 10% loading, carbomer proved to be the best mucoadhesive polymer for silica-loaded CBD.

3.2.2. In Vitro Release of CBD-Loaded Mucoadhesive Carrier Systems

The drug release profiles for the carrier systems compared to pure CBD are shown in Figure 4. Noticeably, the dissolution of CBD from the mesoporous carrier systems was distinctly faster in comparison to the poorly soluble crystalline CBD. For CBD loaded into Aeroperl 300, a 100% release was detected after 60 min. The dissolution profile of pure crystalline CBD on the other hand only reached a value of 40% after 120 min. The enhanced dissolution rate of loaded molecules compared to the pure crystalline drug can be attributed to surface or crystallinity effects [20]. It is understood that the amorphous form, resulting from the loading of CBD into the mesoporous silica, exhibits altered thermodynamic properties and therefore higher dissolution rates than the crystalline phase [20,25,26]. The

presence of the mucoadhesive polymers appeared to decrease the overall rate of CBD release. None of the mucoadhesive carrier systems were able to reach a release of 100% CBD within the 120 min time period studied. Compared to the other carrier systems, the carrier systems containing carbomer exhibited the most pronounced delay with a release of 45% after 120 min, followed by the carrier system with HPMC releasing approximately 60% within 120 min. The release profile obtained for the carrier system with chitosan showed a higher dissolution rate in the first 15 min compared to all other carrier systems. However, the release curve flattened rapidly thereafter and only about 85% CBD was released after 120 min. The observed differences in the release profiles of the mucoadhesive carrier systems can be explained by the different gel-forming ability of the polymers, which slows the release rate of the drug. At the given pH of the artificial saliva (pH 6.9), chitosan possesses very poor gel-forming properties [27], which explains the comparatively higher dissolution rate. The swelling ability of carbomer is superior to that of HPMC, resulting in stronger gel formation and thus CBD encounters greater resistance to diffusion through the carbomer gel layer [28].

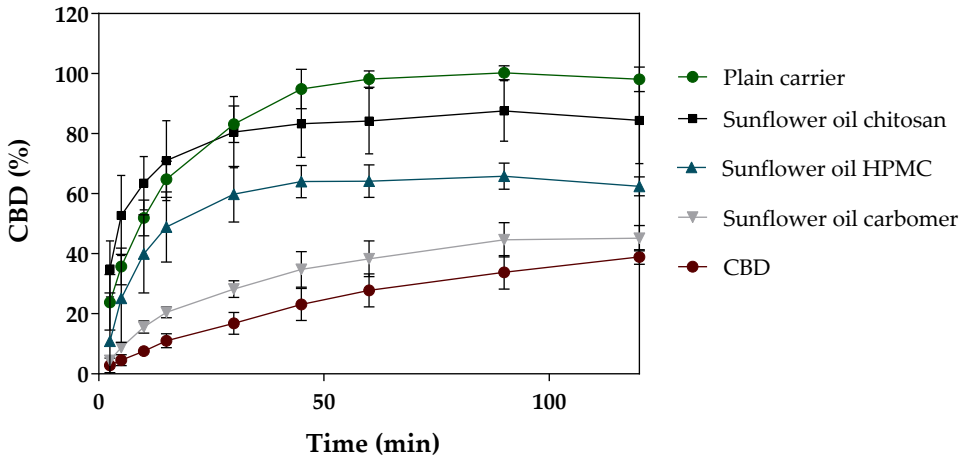


Figure 4. Dissolution profile of pure CBD, CBD incorporated into Aeroperl 300 (Plain carrier) and mucoadhesive carrier systems with chitosan, HPMC and carbomer; n = 3; mean ± SD.

3.2.3. Mucopenetration Studies of CBD-Loaded Mucoadhesive Carrier Systems

Inadequate drug passage through the buccal mucosa can be a major limitation in the administration of transmucosal drug-delivery systems [10]. Therefore, the amount of CBD that penetrated into the mucosa from the carrier systems during the mucoadhesion test was determined. Figure 5 illustrates the amount of CBD that penetrated from carrier systems with and without added mucoadhesive polymer in comparison to pure CBD as a reference. It is noteworthy that a prolonged contact time of CBD on the mucosa did not directly lead to an improved penetration. While incorporating CBD into the mesoporous Aeroperl 300 resulted in an increased amount of CBD penetration compared to pure CBD, the additional incorporation of HPMC and carbomer reduced this effect. In the case of the carrier system with carbomer, the result of the penetration study was even inferior to the reference with pure CBD. Solely the carrier system with chitosan resulted in an increased amount of CBD in the mucosa, indicating that chitosan promotes the mucosal penetration. Compared to pure CBD, the penetrated amount for the chitosan carrier system was 3-fold higher. The increased transmucosal absorption of CBD from the silica carrier systems can be explained by the altered thermodynamic state of CBD in the mesoporous silica, which favors the penetration process [29]. The swelling behavior of the polymers HPMC and carbomer resulted in a hydrophilic gel layer which hinders diffusion of the lipophilic CBD, thus hindering the penetration of CBD into the mucosa. Carbomer forms a stronger gel compared to HPMC [28], as already evident in the release studies, which caused the comparatively lower penetration. The release studies showed that the swelling behavior of chitosan, compared to the other two polymers, did not hinder the release of CBD from

the carrier in the first 15 min. On the contrary, the initial release rate was even slightly increased compared to the polymer-free silica carrier system, which may have contributed to the improved penetration. In the literature, studies with chitosan have demonstrated the enhancing effect on drug penetration through various mucosal tissues including the buccal mucosa [30]. However, the mechanism of penetration enhancement through the mucosa of the oral cavity has not yet been fully understood. The enhancing effect is often attributed to the bioadhesive properties of chitosan [31]. Theories that chitosan interferes with the intercellular organization in the buccal epithelium have yet to be demonstrated [32].

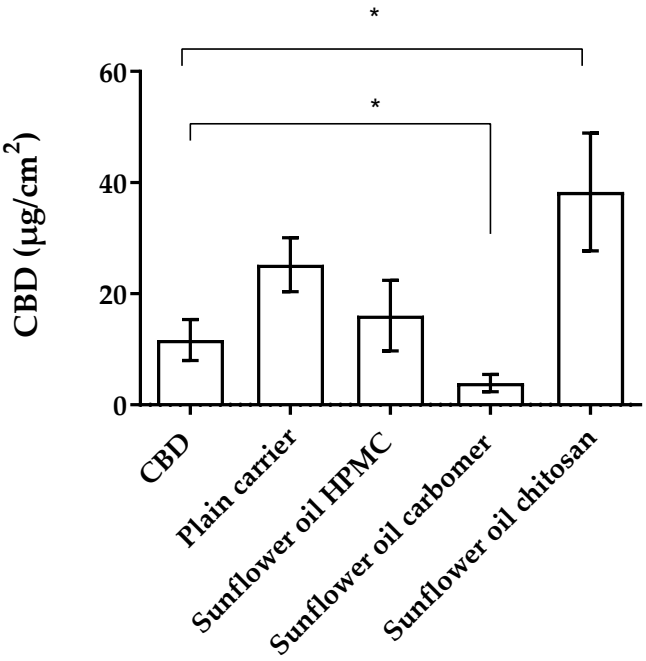


Figure 5. Penetrated amount of pure CBD, CBD incorporated into Aeroperl 300 (Plain carrier) and mucoadhesive carrier systems with chitosan, HPMC and carbomer; * $p < 0.05$, $n = 3$; mean ± SD.

3.3. CBD-Loaded Carrier Systems with Propylene Glykol

To further improve CBD penetration, propylene glycol has been chosen as an additive which has frequently proved to be an effective and orally acceptable penetration enhancer. To this end, CBD-loaded silica carriers containing 2.5, 5, 10 and 20% propylene glycol relative to the total mass were prepared and assessed. The penetration results are represented in Table 2. The addition of propylene glycol resulted in an increase of the penetrated amount in all carrier systems compared to pure CBD. With 2.5% propylene glycol, the amount of CBD in the mucosa was increased by almost 3-fold compared to pure CBD. This represents only a slight increase compared to the respective CBD-loaded silica carrier without propylene glycol. By increasing the propylene glycol concentration, the penetration-promoting effect was further enhanced. As a result, the carrier system with 5% propylene glycol showed a 6-fold increase in penetration relative to pure CBD, which was also a distinct increase relative to the corresponding silica carrier system without propylene glycol. Similarly, high penetration, with penetration enhancement ratios of about 6, were observed for the carrier systems with 10% and 20% propylene glycol. This indicates that 5% propylene glycol is almost sufficient for the greatest possible effect and that a further increase in concentration is not recommended.

Table 2. Penetrated amount of CBD and PE of CBD-loaded silica carriers with different amounts of propylene glycol and CBD as a reference; n = 3; mean ± SD.

Formulation	Penetrated CBD [µg/ cm^2]	PE Ratio
CBD	11.70 ± 3.70	1.00
Aeroperl 300 CBD 0% Propylene glycol	25.23 ± 4.85	2.16
Aeroperl 300 CBD 2.5% Propylene glycol	34.01 ± 8.90	2.91
Aeroperl 300 CBD 5% Propylene glycol	70.47 ± 15.63	6.02
Aeroperl 300 CBD 10% Propylene glycol	72.99 ± 11.78	6.24
Aeroperl 300 CBD 20% Propylene glycol	77.95 ± 6.44	6.66

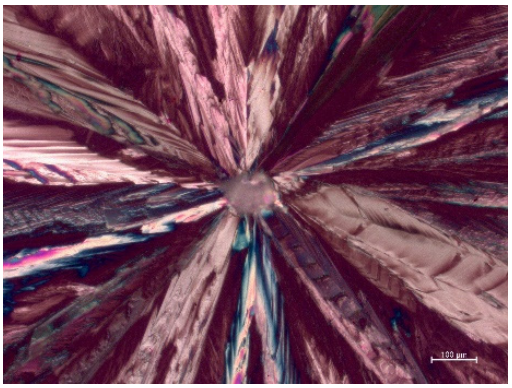
The positive effect of propylene glycol might be attributed to an altered physical state of CBD after loading into the mesoporous silica. Concerning this, different mixtures of CBD and propylene glycol were prepared, dissolved with ethanol, and examined microscopically and by DSC after ethanol was evaporated. The mixtures contained propylene glycol and CBD in ratios of 1:4, 1:3; 1:2 and 1:1, corresponding to the ratios of propylene glycol and CBD in the carrier systems studied with 2.5, 5, 10 and 20% propylene glycol. In the microscopic images (Figure 6), crystals of CBD were clearly visible for the mixtures with the lower concentrations of propylene glycol (ratio 1:4, 1:3 and 1:2), indicating that these concentrations of propylene glycol were not sufficient to completely dissolve the respective amount of CBD. At a ratio of 1:1, the entire CBD was dissolved in propylene glycol as no CBD crystals were microscopically detectable. The corresponding DSC thermographs display the melting events of the CBD–propylene glycol mixtures (Figure 7) in comparison with pure CBD. Pure CBD exhibited a melting range from 66 to 67 °C. Comparatively, the melting events of the blends were shifted to lower temperatures, caused by the interaction of CBD with propylene glycol. While at a ratio of 1:4 the endothermic melting peak is still quite large and broad, the detected melting events became smaller as the concentration of propylene glycol increased, indicating that the crystalline fraction of CBD diminishes. The microscopic observation and the DSC measurement confirm that propylene glycol acted as a co-solvent, and by increasing the concentration of propylene glycol, the dissolved fraction of CBD could be increased. In the respective carrier systems, however, a further increase in propylene glycol above a concentration of 5% did not result in an enhanced penetration. This suggests that the penetration enhancing effect of propylene glycol is not directly linked to its action as a co-solvent in the carrier system and does not require complete dissolution of CBD. Therefore, it is likely that another effect of propylene glycol besides solubilization came into play and affected mucosal uptake of CBD. The mechanism of action of propylene glycol in enhancing drug penetration is not fully understood. In addition to affecting the drug solubility in the vehicle, it is suggested in literature that propylene glycol increases permeant partitioning into and solubility within the intercellular lipids [33].

3.4. CBD-Loaded Mucoadhesive Carrier Systems with Propylene Glykol

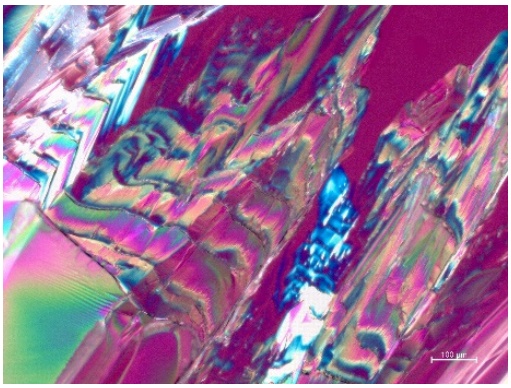
Mucoadhesive carrier systems were prepared with 5% propylene glycol to assess whether the addition of propylene glycol also has a penetration enhancing effect in the carrier systems containing a mucoadhesive polymer. Figure 8 displays the penetrated amount of CBD from the carrier systems with and without propylene glycol. The corresponding penetration enhancement ratios are listed in Table 3. The effect of propylene glycol was most pronounced in the polymer-free carrier system, with a 6-fold increase in mucosal absorption. The addition of propylene glycol also increased penetration from carrier systems with carbomer and HPMC, although these were comparatively less intense. In terms of the penetration enhancement ratio, the carrier with HPMC achieved a 2-fold higher penetration value compared to pure CBD. The carrier system with carbomer showed a distinctly reduced penetration (PE 0.33) due to the polymer addition, which could be increased again to a value (PE 1.20) comparable to pure CBD by using propylene glycol as

enhancer. Consequently, propylene glycol recompensated the penetration-inhibiting effect of carbomer. However, for the carrier system with chitosan, no effect could be observed with the addition of propylene glycol. Both carrier systems, the one with and the one without propylene glycol, exhibited a penetration enhancement ratio of about 3. This suggests that the penetration enhancing effects of chitosan and propylene glycol are not additive. Moreover, these results indicate that co-solvency plays a minor role in respect to penetration enhancement of CBD.

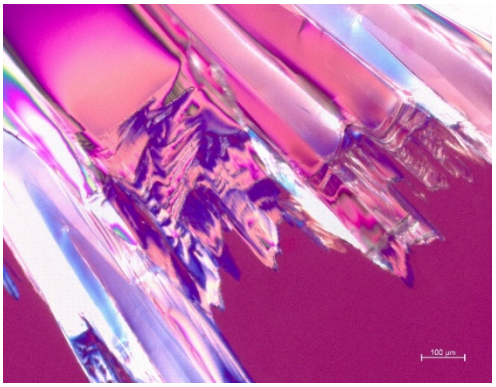
Propylene glycol:CBD (1:4) corresponding to 2.5% (m/m) propylene glycol in the carrier system



Propylene glycol:CBD (1:3) corresponding to 5% (m/m) propylene glycol in the carrier system



Propylene glycol:CBD (1:2) corresponding to 10% (m/m) propylene glycol in the carrier system



Propylene glycol:CBD (1:1) corresponding to 20% (m/m) propylene glycol in the carrier system

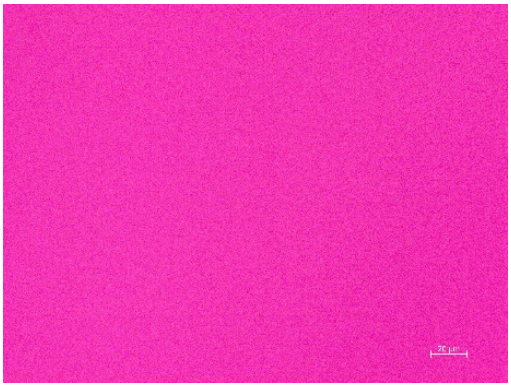


Figure 6. Microscopic images of mixtures of propylene glycol and CBD with the ratio of 1:1, 1:2, 1:3 and 1:4.

Figure 9 illustrates the mucoadhesion kinetic of the carrier systems with propylene glycol. By direct comparison with the mucoadhesion values of the carrier systems without propylene glycol (Figure 3), it can be clearly seen that the addition of propylene glycol had almost no effect on the mucoadhesive properties of the carrier systems.

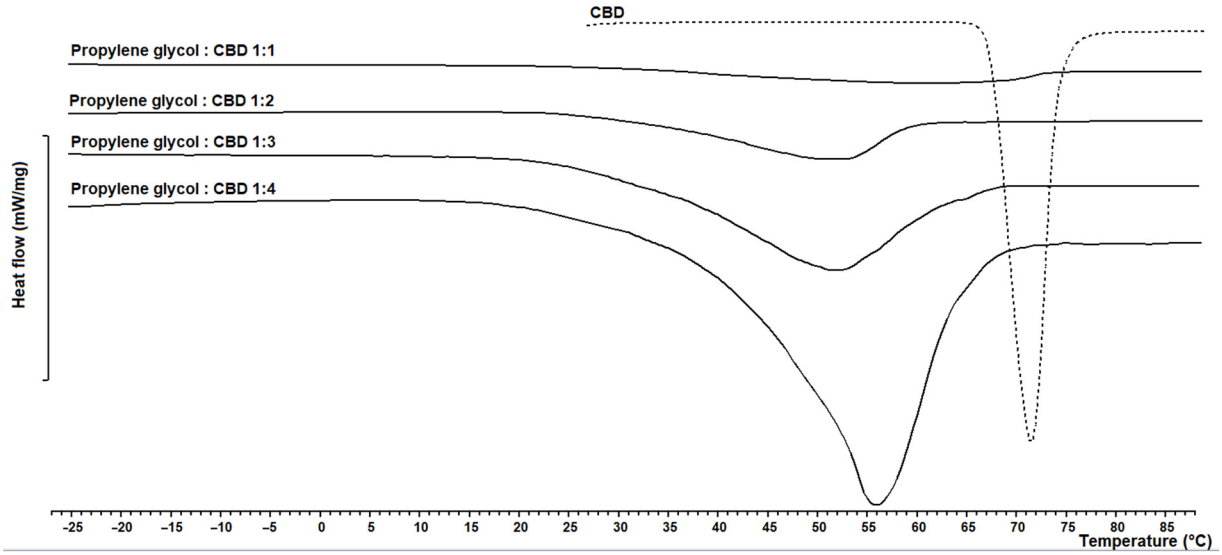


Figure 7. Comparison of DSC curves obtained by the mixture of propylene glycol and CBD with the ratio of 1:1, 1:2, 1:3 and 1:4.

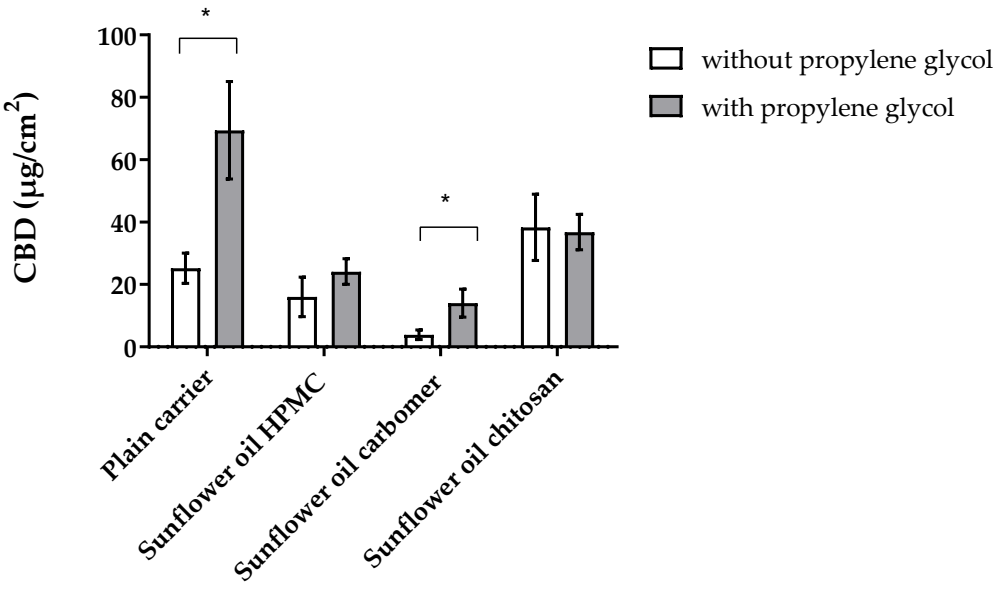


Figure 8. Comparison of the penetrated amount of CBD from carrier systems with and without propylene glycol (Plain carrier: CBD-loaded polymer-free carrier systems); * $p < 0.05$, $n = 3$; mean ± SD.

Table 3. Comparison of penetration enhancement ratios of carrier systems with and without propylene glycol and pure CBD as a reference (Plain carrier: CBD-loaded polymer-free carrier system); $n = 3$; mean ± SD.

Formulation	without Propylene Glycol		with Propylene Glycol	
	Penetrated CBD [µg/cm^2]	PE	Penetrated CBD [µg/cm^2]	PE
CBD	11.70 ± 3.70	1.00	-	-
Plain carrier	25.23 ± 4.85	2.16	69.39 ± 15.62	5.93
Sunflower oil HPMC	16.06 ± 6.35	1.37	24.15 ± 4.14	2.06
Sunflower oil carbomer	3.91 ± 1.54	0.33	14.06 ± 4.52	1.20
Sunflower oil chitosan	38.31 ± 10.81	3.27	36.79 ± 5.67	3.14

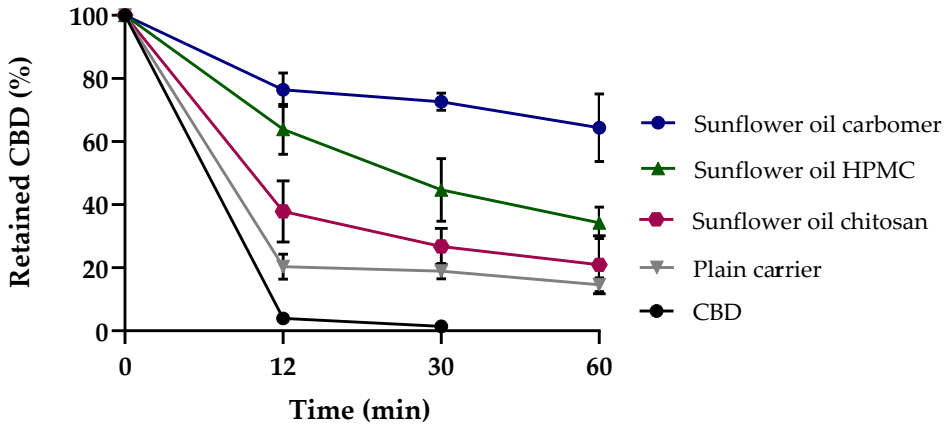


Figure 9. Mucoadhesion kinetic of CBD-loaded mucoadhesive carrier systems with propylene glycol and pure CBD as a reference (Plain carrier: CBD-loaded polymer-free carrier system; n = 3; mean ± SD.

4. Conclusions

Supplementing CBD-loaded silica carrier systems with mucoadhesive polymers from a suspension in sunflower oil proved to be an efficient strategy for prolonging the residence time of CBD at the buccal mucosa and minimizing drug loss due to the washing effect of saliva. Of all tested carrier systems, the one containing carbomer proved to be superior, not only regarding the intensity of mucoadhesion, but also with respect to the duration of the effect. However, HPMC and chitosan were also able to considerably improve mucoadhesion compared to pure CBD, increasing by 16-fold and 8-fold, respectively. The improved contact of the formulation with the mucosa did not directly lead to improved penetration. HPMC and carbomer strongly swell upon contact with saliva and thus hinder both the release of CBD from the carrier system and subsequently its penetration into the mucosa. By contrast, chitosan, as a mucoadhesive polymer, exhibited a penetration enhancing effect and was able to increase the absorbed amount of CBD by about three times compared to pure CBD. Therefore, chitosan represents a suitable and biocompatible option for the development of transbuccal delivery systems with sufficient mucoadhesive properties and improved penetration, without relying on the addition of penetration enhancers. Except for the carrier systems with chitosan, where the addition of propylene glycol showed no further penetration-promoting effect, propylene glycol increased the mucosal absorption of CBD in all other carrier systems without affecting the mucoadhesive properties. In the case of the carrier system without a mucoadhesive polymer, 5% propylene glycol improved the penetrated amount by as much as 6-fold. For the carrier system with HPMC, twice the amount of CBD penetrated into the buccal mucosa after adding 5% propylene glycol. Regarding the carrier system with carbomer, the addition of propylene glycol compensated the penetration hindering effect of the mucoadhesive polymer and increased the penetrated amount of CBD to a level slightly above the reference value of pure CBD.

Overall, the data obtained confirms that the approach of using CBD-loaded silica carriers and optimizing them with mucoadhesive polymers and penetration enhancers as needed is a promising strategy for the development of buccal drug delivery systems. In this way, the advantages of oromucosal routes of administration can be exploited with the potential to enhance the therapeutic effect of CBD.

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