# Improved permeability of acyclovir: Optimization of mucoadhesive liposomes using the PVPA model

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## ABSTRACT

The antiviral drug Acyclovir (ACV) suffers from poor solubility both in lipophilic and hydrophilic environment, leading to low and highly variable bioavailability. To overcome these limitations, this study aimed at designing mucoadhesive ACV-containing liposomes to improve its permeability. Liposomes were prepared from egg phosphatidylcholine (PC) and egg phosphatidylcholine/egg phosphatidylglycerol (PC/PG) and their surfaces coated with Carbopol. All liposomal formulations were fully characterized and for the first time the PVPA model was used for testing *in vitro* permeability of drug from mucoadhesive liposome formulations. The negatively charged PC/PG liposomes could encapsulate more ACV than neutral PC liposomes. Coating with Carbopol increased the entrapment in the neutral PC liposomes. Incorporation of ACV into liposomes exhibited significant increase in its *in vitro* permeability, compared to its aqueous solution. The neutral PC liposomal formulations exhibited higher ACV permeability values compared to charged PC/PG formulations. Coating with Carbopol significantly enhanced the permeability from the PC/PG liposomes, as well as sonicated PC liposomes, which showed the highest permeability of all tested formulations. The increased permeability was according to the formulations' mucoadhesive properties. This indicates that the PVPA is suitable to distinguish between permeability of ACV from different mucoadhesive liposome formulations developed for various routes of administration.



**Key words**

Acyclovir, permeability, *in vitro* model, liposomes, artificial membrane, lipid, Carbopol, mucoadhesive


**List of abbreviations**

ACV, acyclovir

E-80, egg phospholipids

E-PC, egg phosphatidylcholine

E-PG-Na, egg phosphatidylglycerol sodium

HPLC, high performance liquid chromatography

HSV-1, human *Herpes simplex* virus type-1

HSV-2, human *Herpes simplex* virus type-2

PAMPA, parallel artificial membrane permeability assay

PB, phosphate buffer

PBS, phosphate buffer saline
PCS, photon correlation spectroscopy
PVPA, phospholipid vesicle-based permeation assay
STDs, sexually transmitted diseases

## INTRODUCTION

Acyclovir (ACV) is a commonly used antiviral agent despite the fact that most of its currently available dosage forms, i.e. tablets, suspension, cream, fail to achieve suitable levels at targets sites following oral, local or parenteral administration.[1] Its absorption from GI tract is slow and incomplete and oral bioavailabilities ranges from only 10 to 30 %. As a consequence, higher doses are prescribed, resulting in systemic toxicity and adverse reactions.[2] The current therapy with ACV intended for local vaginal therapy is also limited to the conventional dosage forms, namely tablet and cream. However, also in local therapy, its bioavailability is low and highly variable which requires a higher administration frequency. In addition, topical administration of ACV, although preferable in pregnant patients, is associated with several disadvantages such as low retention at the vaginal epithelium, messiness, and poor patient compliance.[3,4]

The development of an efficient formulation of ACV is of increasing importance since, in spite of the continuous efforts to raise the awareness of the increased incidence of sexually transmitted diseases (STDs), their prevalence is increasing resulting in an increased concern related to the limitations of currently available therapies. Among various STDs, the infections with human *Herpes simplex* virus type-1 and type-2 (HSV-1 and HSV-2) remain among the most common.[5] Particularly worrying are the recent findings by Avalos and colleagues[6] suggesting that there is the increased prevalence of women delivering an infant exposed to antiviral medication over time. The need for the assessment of the safety and effectiveness of antiviral medications during pregnancy is therefore of most importance.

Acyclovir, a class III drug according to BCS classification[2], is characterized by its hydrophobicity and low solubility both in water and in lipid bilayers. The bioavailability of such drugs suffering from limited solubility and permeability could be optimized and improved by controlling the drug carrier's properties such as nanoparticle size and surface characteristics.[7]

Particularly interesting as drug carriers are mucoadhesive nanopharmaceuticals, expected to enhance the contact time between the delivery system and absorbing membrane and maintain a concentration gradient between the drug to be absorbed and the tissue, providing the enhanced delivery of the drugs to the underlying tissue, a prerequisite for the successful anti-infective therapy.[8] In vaginal therapy, the high drug concentrations in genital tissues are desirable, as this biological compartment is the relevant target site for ACV administered intravaginally.[9] In addition, through the use of mucoadhesive polymers such as poly(acrylic acid) derivatives and chitosan in the preparation of delivery systems, it is also possible to act on increased epithelial permeability of many poorly permeable drugs.[10]

To determine the permeability characteristics of drugs and drug formulations an efficient permeability model is required. A novel screening model for passive drug permeability, the phospholipid vesicle-based permeation assay (PVPA), was developed by us to mimic the intestinal epithelia.[11] Recently, also a modified PVPA mimicking healthy and compromised skin barrier has been introduced.[12] The original PVPA barriers are prepared by depositing liposomes from egg phosphatidylcholine onto a filter support through centrifugation followed by a freeze-thaw cycling to promote fusing of liposomes and to obtain a tight barrier. The PVPA has been successfully used in permeability testing of the marketed drug substances, novel active substances as well as drug in complex formulations, and has shown potential to automation using a robotic system with a connected plate reader.[13-16] The functionality of the barriers has shown to be stable and the barriers retained their integrity within a pH range from 2.0 to 8.0.[17] Accordingly, the PVPA has proven to be suitable to both obtain information on segmental absorption in the gastrointestinal tract as well as have potential to work as permeability model for absorption sites with a broad range of pHs in their environment. Further, since the PVPA barrier consist mainly of phosphatidylcholine, a component found in many absorption barriers; it has the potential to serve as a general model mimicking other absorption barriers.

The aim of this study was to improve permeability of ACV through developing and optimizing mucoadhesive liposome formulations. In order to assure liposomal retention on the mucosal site, liposomes were coated with mucoadhesive polymers (Carbopol® or chitosan respectively). This approach was expected to prolong the residence time at the administration site and improve the release profile of incorporated ACV. As ACV can be applied through various routes of drug administration, we applied the well-established PVPA model developed in our laboratory, as a tool in optimization of liposome characteristics and drug permeability profile.

## MATERIALS AND METHODS

### Materials

Acetic acid (glacial) anhydrousGR, dinatriumhydrogenphosphat-dihydrat, chloroform, potassium chloride were purchased from Merck (Darmstadt, Germany). Acycloguanosine, potassium phosphate monobasic, sodium chloride, methanol CROMASOLV®, chitosan low molecular weight (LMW), Mucine from porcine stomach Type II were obtained from Sigma-Aldrich (Steinheim, Germany). Lipoid E-80 (egg phosphatidylcholine, 80% phosphatidylcholine), Lipoid S 100 (soybean lecithin, >94% phosphatidylcholine) and Lipoid E PG-Na (egg phosphatidylglycerol sodium) were obtained from Lipoid (Ludwigshafen, Germany). Carbopol®974P NF was purchased from Noveon Inc. (Cleveland, USA). Poloxamer 188, Pluronic® F68 NF Prill was product of BASF Corporation (New Jersey, USA). Dialysis membrane MWCO 12-14000 Daltons was obtained from Medicell International Ltd. (London, UK).

Filter inserts (Transwell, $d = 6.5$ mm) and plates were purchased from Corning Inc., (Corning, New York, USA). The mixed cellulose ester filters (0.65 µm pore size) and the isopore filters (0.8 µm pore size) were obtained from Millipore (Billerica, Massachusetts, USA). Whatman® nucleopore track-etch membrane filters (0.4 µm pore size) were obtained from Whatman (part of GE Healthcare, Oslo, Norway).

### Quantification of ACV

ACV was quantified by the reversed phase high performance liquid chromatography (HPLC) with a reverse phase column C18 (5 µm; 3.9x150 mm Waters, Milford, MA, USA) on a Waters Photodiode Array Detector HPLC equipped with a UV detector (Waters, Milford, MA, USA). The mobile phase consisted of MeOH: MilliQ water (1:1 v/v), pH 2.5 and the flow rate was 0.4 ml/min. ACV was detected at wavelength 258 nm, and the measuring time adjusted to 7 minutes. The sample concentration was determined from a standard curve made by diluting known amounts of ACV in both water and methanol. To exclude interference due to similar retention time, separate measurements for lipid and polymer solutions namely, phosphatidylcholine (Lipoid S100), phosphatidylglycerol (PG), polymers (Carbopol®, chitosan) and solvents (Poloxamer 188, water, methanol) were performed. All measurements were done in triplicates. The calibration curve resulted in good linearity in the wide

concentration range 0.5-50 μg/ml with $R^2$ of 0.9997 and 1.0000 in water and methanol, respectively.

## Preparation of ACV-containing Liposomes

Liposomes containing ACV were prepared by the film hydration method and two compositions of lipids were used: PC (Lipoid S-100) and PC/PG (9:1, weight ratio) (Table 1). The lipid components (200 mg, total lipid) and ACV (20 mg) were dissolved in methanol in a round bottom flask. The organic solvent was removed on a rotary evaporator under vacuum of 55 hPa at 45 °C for a period of 1 hour to remove all traces of the solvent. The dried lipid film was than hydrated with 10 ml of distilled water to obtain liposome dispersion. The liposomes were stored in refrigerator for at least 24 hours for further use and characterization.

In order to achieve smaller and more uniformly distributed vesicles, the liposomal dispersions were sonicated by Sonics high intensity ultrasonic processor (Sonics & Materials Inc., Newtown, USA) for 1 minute. The amplitude setting was 500 Watt/20 kHz processor 40 %. Upon sonication, the liposomal suspensions were placed in refrigerator at 4 °C overnight before further experiments were performed.

## Coating of Liposomes

The ACV-containing liposomes, both non-sonicated and sonicated, were coated with polymers (chitosan and Carbopol) in the presence of unentrapped ACV. The 0.1% and 0.6% (w/v) chitosan solutions were prepared in 0.1% (v/v) glacial acetic acid. The 0.1% (w/v) Carbopol®974P NF solution was prepared by dissolving Carbopol in PBS, pH 7.4. The chitosan or Carbopol solutions (2.0 ml) were added drop-wise to 2.0 ml of liposomal suspension under magnetic stirring at room temperature for 1 hour. The coated liposomal suspensions were then placed in refrigerator overnight to stabilize. For all preparations the rate of stirring was kept constant.[18]

## Characterization of ACV-Containing Liposomes

### *Particle size*

The mean particle size and size distribution of the liposomes were determined by photon correlation spectroscopy (PCS) using a Submicron Particle Sizer 370 (PSS Nicomp Particle Sizing Systems, Santa Barbara, CA, USA). Sample preparation and measuring conditions were as described earlier.[19] Three cycles of 15 minutes measurements were performed.

***Entrapment Efficiency***

The entrapment efficiency for the ACV-loaded liposomes was determined using dialysis tubing (Medicell International Ltd., London, UK) with a MWCO 12-14000 Da as the separation method.[18] Samples of liposomal suspensions (1 ml) were filled into dialysis tubes and dialysed against distilled water for 6 hours. The volume of medium was adjusted to assure the solubility of ACV. Liposomal samples were diluted with MeOH to dissolve the lipids and liberate the drug. After dialysis ACV, both liposomally-associated (liposomes inside the dialysis tube disintegrated in methanol) and unentrapped found in dialysis medium, were determined by HPLC, as previously described.

***Zeta Potential Determination***

Zeta potential measurements were performed on a Malvern Zetasizer nano Z (Malvern, Worcestershire, UK). The liposomal suspensions were diluted in 1:40 ratio in filtrated water before measurements. The results are the average of at least three independent formulations.

***Mocoadhesion properties measured by in vitro interactions with pig mucin***

Mucoadhesion activities of the liposomes were determined utilizing the method reported by Pawar and colleagues.[20] The binding activity of liposomes was determined by mixing pig mucin (PM) suspension in PBS, 0.05M, pH 7.4 (400 µg/ml) with liposomes (1:1 v/v). After incubation at room temperature for 2 hrs, the samples were centrifuged at 216 000 g for 60 min (Optima LE-80, Beckman, USA). The absorbance of the remaining free PM in the supernatant was determined at by UV spectrophotometer at λ=251 nm.

The PM binding efficiency of liposomes was calculated from the following equation:

$$\text{PM binding efficiency} = \left(\frac{C_0 - C_s}{C_0}\right) \times 100 \quad (1)$$

where $C_0$ is the initial concentration of PM used for incubation and $C_s$ is the concentration of free PM in the supernatant.

**Preparation of PVPA Barriers**

The liposomes used for the PVPA barriers were prepared by the film hydration method. Egg phosphatidylcholine was dissolved in a mixture of chloroform and methanol (2:1 v/v) in a round bottom flask. The organic solvents were removed on a rotary evaporator under vacuum of 55 hPa at 45 °C for a period of 3 hour to remove all traces of the solvent. The lipid film were then hydrated with phosphate buffer (PB) pH 7.4 and 10% (v/v) ethanol to obtain a 6 % (w/v) liposomal dispersion as described earlier.[11] The liposomal dispersion was further extruded by hand using syringe holders containing filters with pore size 0.4 and 0.8 μm, respectively in order to obtain dispersions with two different size distributions.

The PVPA barriers were prepared in accordance to procedure described earlier by us.[11] Briefly, the liposome dispersions with two different size distributions were deposited on cellulose ester filter inserts by centrifugation to obtain a tight barrier. In more details smaller liposomes, which had been finally extruded through 0.4 μm polycarbonate membrane filters, were added first and the inserts centrifuged at 600 *g* for the liposomes to enter into the pores of the filter. The inserts were then kept in an incubator at 50°C for 45 min to partly evaporate the water and ethanol. The bigger liposomes, extruded through 0.8 μm polycarbonate membrane filter, were then added and the inserts centrifuged at 600 *g* for 30 min for the liposomes to layer on top of the filter. Upon invert centrifugation to remove excess buffer the barriers were stored at -80°C and then heated at 65°C for 30 minutes right before conducting the permeation experiments. After the last heating step the barriers appear with an even finish and no wet spots are visual, but earlier characterization has shown that the barrier is partly hydrated at this stage.[21]

### *In vitro* Permeability Studies on the PVPA

The permeability of ACV from the different liposome formulations was determined using the PVPA model. Permeability experiments were performed at room temperature after loading the inserts with 100 μl previously dialyzed liposomal preparation or ACV solutions and placing the inserts in acceptor wells containing acceptor solution (500 μl). These experiments were performed according to the procedures described earlier by Flaten and co-workers,[11] only that the acceptor solution was upgraded from PBS (pH 7.4) to a more complicated system containing Poloxamer 188 solution (2 mg/ml, pH 7.4). The inserts were further moved at certain time intervals over a period of 5 hours to new acceptor wells with fresh acceptor solution to ensure sink conditions. At the end of the permeation experiments, samples (200 μl) from each acceptor compartment were analyzed by using HPLC as described above. The experiments were performed at least in triplicate, with 6 inserts in each parallel and the apparent permeability coefficient ($P_{app}$) of the different formulations was calculated using the following equation:[11]

$$P_{app}(cm/s) = \frac{J}{A(C_d - C_a)} \tag{2}$$

where J is the observed flux rate under steady-state conditions (nmol/s), A is the surface area of the inserts ($cm^2$), $C_d$ and $C_a$ are the concentrations of compounds in donor and acceptor chambers (nmol/mL), respectively. Since the experiments were performed under sink conditions, the equation could thus be simplified to:

$$P_{app}(cm/s) = \frac{J}{AC_d} \tag{3}$$

Following the permeations studies, the electrical resistance of the barriers was measured (Millicell-ERS, Millipore, USA) to confirm the integrity of the barriers.

**Statistical Methods**

To identify significant differences between two sets of data the Student's *t*-test for comparison of two means was performed. The experiments were performed in triplicates unless otherwise stated, and a significance level of $p < 0.05$ was always used.

## RESULTS AND DISCUSSION

Over the past thirty years, liposomes have received much attention as drug carriers for improving absorption of poorly absorbed drugs such as ACV. Recently, several attempts have been made to enhance ACV bioavailability by including mucoadhesive excipients in different drug formulations with the main focus on natural and biocompatible products.[3,4,22,23] The polymers such as chitosan and Carbopol are well known excipients with mucoadhesive properties which are considered to have potentials to improve both intestinal and vaginal absorption. Their application results in increased retention time at the administration site, resulting in increased bioavailability and improved therapy.[24]

**Characterization of ACV-containing Liposomes**

We investigated the effect of lipid composition and type of polymer used in coating as means to optimize mucoadhesive liposomes containing ACV. Alsarra et al. (2008) suggest that ACV is present in its neutral form when dissolved in the organic solvent, and interacts with phospholipids in the organic phase before the lipid film formation. As the aqueous solution is added to the dry film, the hydrophobic interactions between ACV and the phospholipids become more effective. Upon the hydration, more drug molecules becomes encapsulated within the liposomes.[23] By using this approach, the higher entrapment efficiency, as determined for our liposomes, corresponded well to the results reported by Alsarra and colleagues.[23]

Sonication of liposomes reduces the size and leads to a more uniform size distribution.[25] However, it also simultaneously causes a significant loss of entrapped drug. We have applied the polymer coating in the presence of unentrapped ACV in order to increase the entrapment efficiency.[18] However, the coating of liposomes with chitosan (both concentrations) failed to improve the ACV entrapment efficiency and resulted in rather low entrapment values after the sonication of vesicles (approximately 3%, data not included). The chitosan-coated liposomes were therefor not included in the further studies. A possible hypothesis for this unexpected low entrapment efficiency could be due to an interaction between negatively charged lipid and positively charged chitosan forcing ACV out of the bilayer. However, further investigate is needed to elucidate the mechanism behind this observation. Coating with 0.1% (w/v) Carbopol solution performed in the presence of unentrapped drug did however enhance the entrapment efficiency (Table 2) according to previously findings.[18] Carbopol-coated vesicles were therefore used in all further studies.

Lipid composition of liposomes has been shown to affect the encapsulation of ACV.[4,26,27] The entrapment efficiency of ACV into various non-coated and coated liposomal formulations was therefore evaluated using different lipid compositions applying Carbopol-coating. The results, presented in Figure 1/Table 2, indicate that neutral PC liposomes could encapsulate less ACV than the negatively charged PC/PG liposomes. These findings are in agreement with earlier studies.[4,26,27] The higher entrapment efficiency in negatively charged liposomes may probably be due to an interaction between the drug and the negatively charged lipid bilayer. PG is also known to be a stabilizing agent and may exhibit a positive effect on entrapment of drugs in liposomes.[4] However, coating of liposomes had an influence on the encapsulation of ACV into the neutral PC liposomes only. In general, entrapment efficiency is known to be higher in

polymer-coated liposomes if the coating is performed in the presence of unentrapped drug. [18,28] Liposomes made of pure PC behaved in expected manner and the entrapment efficiency of ACV in non-coated and coated PC liposomes were found to be 15.24 ± 1.85 % and 20.34 ± 5.43 %, respectively. However, in preparations containing PG, an additional coating with Carbopol did not improve the entrapment efficiency which may be the consequence of the presence of charged lipid in the bilayer and polymer on the surface, both being negatively charged (Table 2). Even though coating increased the entrapment of ACV in PC liposomes there was no significant difference between entrapment efficiency in coated PC liposomes compared to coated PC/PG liposomes. Further the sonication resulted in a loss of approximately 50 % of originally entrapped ACV (Figure 1), as observed for both lipid compositions. Summarizing, the highest entrapment of ACV was obtained within non-sonicated coated PC liposomes and non-coated PC/PG liposomes.

The size distribution and surface characteristics of the non-sonicated and sonicated liposomal formulations was measured before and after coating with Carbopol 0.1% (w/v) and the results are given in Table 2. All liposomal suspensions exhibited expected bimodal size distribution,[29] namely two clear vesicles populations could be detected and the mean peaks of each of the populations with the corresponding percentage of vesicles in that size population are presented in Table 2. Neutral liposomes were found to be bigger in size than liposomes composed of PC/PG. In general, neutral liposomes are expected to be larger than charged liposomes, due the higher number of aggregates present in the liposomal suspension which may be measured as single larger vesicles.[4] Both the presence of negatively charged PG and sonication of 1 minute resulted in size reduction and a more homogeneous size distribution, as expected. Size reduction was also observed after coating the liposomes with Carbopol. This might be attributed to a strong charge repulsion effect of Carbopol. In addition, some polymers (e.g. chitosan) are known to form a cage-like steric barrier that protects the liposomes from aggregation. Therefore, the coated liposomes can be smaller than non-coated ones.[30]

The results of the surface characterization showed that introduction of the negatively charged lipid (PG) resulted in higher negative zeta potential values as expected. This thus confirms successful introduction of PG in lipid bilayer.[4,30] Further, the zeta potentials were increasingly negative for the Carbopol-coated liposomes compared to non-coated liposomes, which confirmed formation of coating layer made of an anionic polymer for both neutral and charged liposomes. These findings were also in agreement with earlier publications.[28] Sonicated liposomes on the other hand exhibited reduced negative zeta potential as compared to non-sonicated ones. The reasons may be due to larger total surface area of sonicated liposomes.[30]

The mucoadhesion properties of the formulations were tested by *in vitro* interactions with pig mucin. The results are displayed in Figure 2 and clearly show that the coated PC-formulations were demonstrating higher degree of mucoadhesion than the non-coated PC-liposomes according to what was expected. Small sonicated liposomes with a larger surface area also showed to be more mucoadhesive compared to the non-sonicated bigger liposomes.

For the PC/PG liposomes the trend was not as clear since the non-coated formulation already showed quite high PM binding. The reason for this is not clear at the moment but could possibly be due to electrostatic interactions. However, the degree of PM binding was the same for the PC and PC/PG Carbopol coated formulations, suggesting that it is the coating and not the under laying liposome that is deciding.

### *In vitro* Permeability Studies

The PVPA was selected as a simple *in vitro* model for the evaluation of ACV permeability in this study. This was the first time the PVPA model was used to assess permeability from mucoadhesive liposomal formulations. PVPA is a novel screening model for passive drug permeability that has shown to provide a good correlation of permeation of drug in aqueous solution to *in vivo* data on intestinal permeability to the same extend as the Caco-2-cell model and better than the parallel artificial membrane permeability assay (PAMPA).[11] The model has further been proven to be stable within a broad range of pH values, in addition to be compatible with various surfactants and co-solvents.[31,32] These findings confirm that the model is suitable for rapid screening of passive diffusion of drugs providing an insight to segmental absorption in the human gastrointestinal tract as well as have potential to work as permeability model for absorption sites with different pH in their environment. More complex cell based models like the Caco-2 model has earlier been used to assess the permeability from liposomes and mucoadhesive formulations.[33-35] However since this is an early development of formulations of ACV for both vaginal and intestinal delivery a simpler and less time consuming permeation model was used here.

Eight liposome formulations in addition to ACV solution were tested on the PVPA and their mean $P_{app}$ and electrical resistance values are given in Figure 3. The ACV solution was included for easier comparison of liposomal influence on permeability of ACV *in vitro*. The $P_{app}$ of the ACV solution across the PVPA was found to be $0.22 \pm 0.01 \times 10^{-6}$ cm/s. The permeability of ACV was significantly increased for all the liposomal formulations except from PC/PG-non-coated-sonicated liposomes where there were no significant difference. The neutral PC liposomal formulations exhibited higher ACV permeability values compared to charged PC/PG

formulations. Liposomes containing charged lipids, such as PG has previously been found to enhance the encapsulation efficiency of ACV, probably because of an interaction occurring between the drug and the lipid bilayer.[4] The reason for the lower permeability of ACV from negatively charged liposomes might thus be due to a change in partition coefficient between the liposome formulation and the PVPA barrier favouring the formulation/donor compartment. Coating with Carbopol significantly enhanced the permeability of ACV from the PC/PG liposomes, as well as sonicated PC liposomes. The increase in permeability observed for the sonicated PC liposomes upon coating with Carbopol was in agreement with increased mucoadhesion. For the non-sonicated PC liposomes on the other hand no increase in permeability, as expected from the increase in mucoadhesion, was observed upon coating. The reason for this is most probably that these liposomes are significantly larger (> 1μm) in size compared to the other formulations which could lead to stacking of the liposomes and hence hampering the contact between the barrier and the formulation. For the PC/PG liposomes the increase in permeability upon coating could neither be explained by the change in mucoadhesion observed. Coating did however decrease the negative surface charge and would thus allow a tighter packing/closer contact between the liposomes and between the liposome formulation and the barrier, leading to increased permeability. A clear correspondence between mucin binding and increase in permeability is however not necessarily expected since this is two different model systems for estimation of biological phenomenon. The highest permeability was however found for the coated sonicated PC liposomes with a $P_{app}$ of $2.48 \pm 0.19 \times 10^{-6}$ cm/s. This was also the formulation showing the highest PM binding efficiency of the formulations tested for permeability. Coating of liposomes resulted, as stated before, in smaller mean size of the liposomes. This together with the liposomal mucoadhesive properties and/or reduction of surface charge is probably providing a closer contact between the liposome formulation and the PVPA barriers, and hence leading to increased permeability of ACV. Incorporation of ACV in liposomes thus enhanced the permeability significantly ($p < 0.05$) and the same trend is seen for all ACV containing liposomal formulations. However, the simplest formulations, Carbopol-coated pure PC liposomes, turned out to be the most promising formulation from a permeability point of view.

The electrical resistance measured at the end of the permeability experiments showed resistance values off scale for all the liposomal formulations. By removing the liposomal formulations from top of the barriers and replacing them with PB the values for electrical resistance could however be obtained (Figure 3). A decrease in resistance compared to a control containing buffer was observed, while the electrical resistance measured for the ACV solution resulted in

increased resistance values compared to the control. Low electrical resistance is usually associated with partial dissolution or disruption in the packing of the barrier while high electrical resistance might be due to interactions between components in the formulation or acceptor buffer. It could be speculated that increase in/off scale electrical resistance when the aqueous donor compartment is loaded with liposomal formulations might be due to a change in viscosity.[32] The reduced electrical resistance after replacing the liposome dispersion with buffer on the other hand is most probably due to turbulence in the donor chamber upon removing and replacing donor solution. Since Poloxamer 188 has previously shown not to influence the electrical resistance at concentrations up to 50 mg/ml[32] the high resistance values may indicate an interaction between the drug and the PVPA barriers. This has also been observed in earlier studies where other drugs have interfered with the barriers resulting in increased electrical resistance.[36]

The PVPA has earlier been used to determine the permeability of drugs in formulations e.g. micelles and solid dispersions.[16,32] Here it was shown that the PVPA worked well also for permeability testing of liposomal formulations and is further suitable to distinguish between different mucoadhesive liposome formulations of ACV.

Moreover, since the PVPA barrier has potential to serve as a general model mimic different absorption barriers this shows that liposomes are promising drug delivery systems for ACV through various routes of administration.

## CONCLUSIONS

The PVPA was here used for the first time for testing *in vitro* permeability from mucoadhesive liposome formulations. Incorporation of ACV into liposomes was found to significantly increase its *in vitro* permeability and coating with Carbopol further enhanced the permeability from the PC/PG liposomes, as well as sonicated PC liposomes. The results indicate that the PVPA works well in permeability testing of liposomal formulations and is suitable to distinguish between permeability of ACV from different mucoadhesive liposome formulations developed for various routes of administration. It is thus a straightforward mean to optimize mucoadhesive liposomal formulations also for other drugs as well as for different routes of administration.

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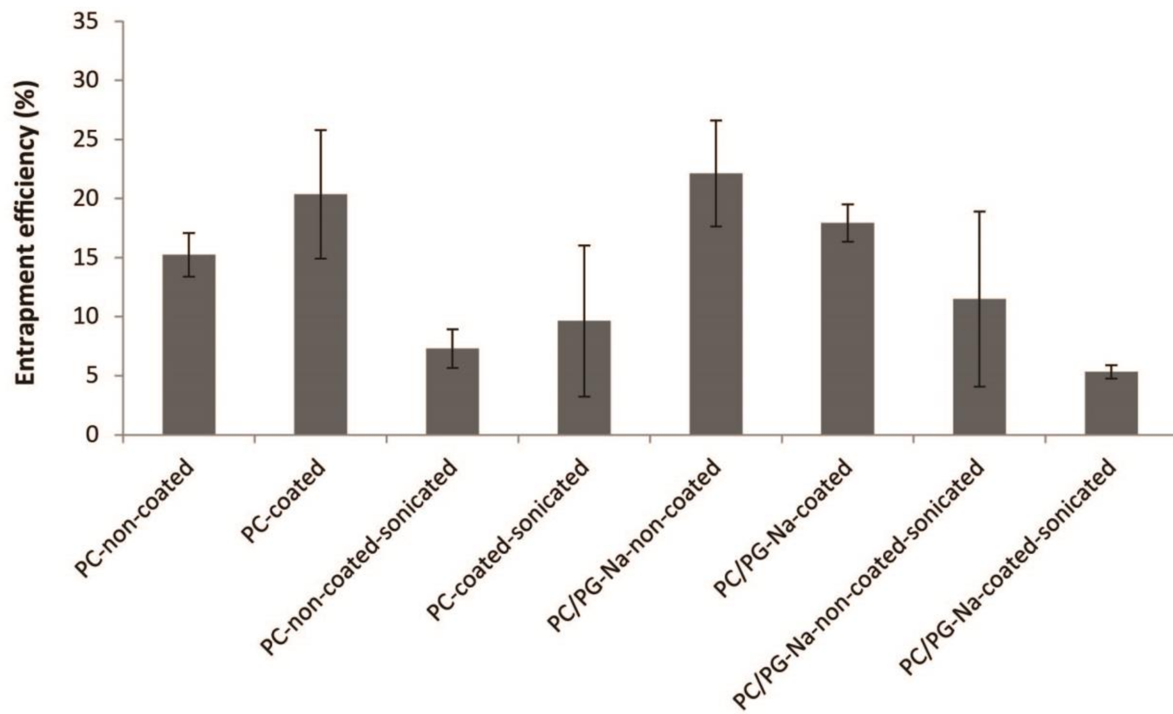


Figure 1. Entrapment efficiency of ACV in the different liposome formulations

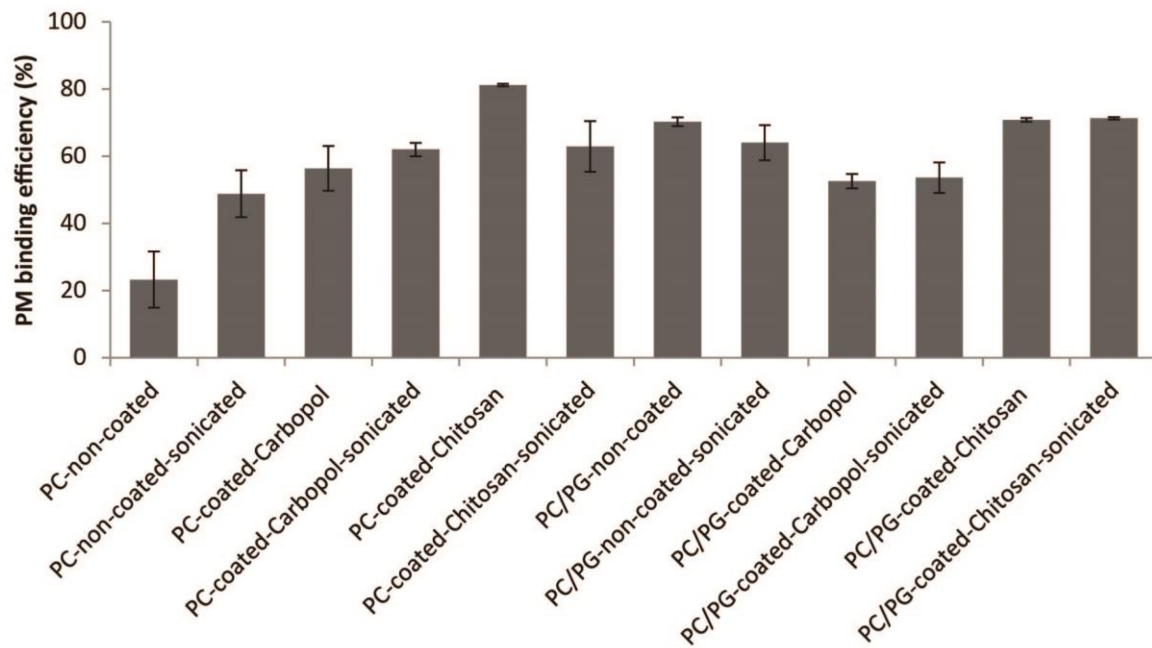


Figure 2. Pig mucin (PM) binding efficiency for the non-coated and coated liposome formulations (n=3)

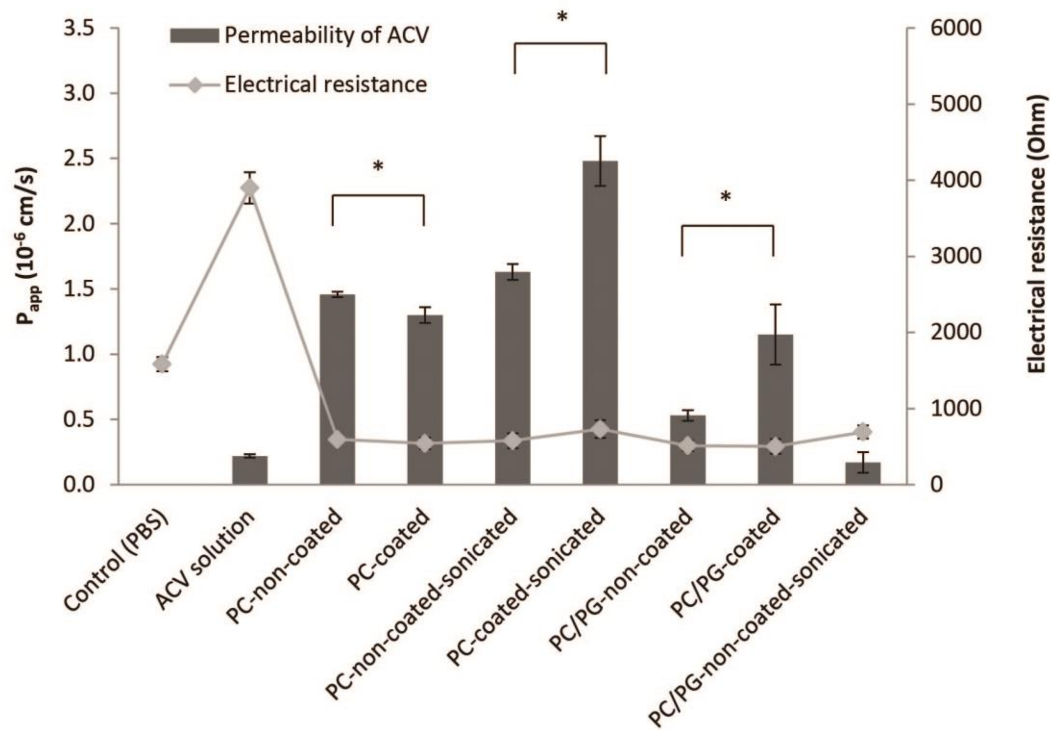


Figure 3. Permeability of ACV from the different liposome formulations together with electrical resistances across the permeation barriers.*$p < 0.05$

Table 1. Liposome composition

| | Composition | | |
|---|---|---|---|
| Formulation | ACV (mg) | PC (mg) | PG-Na (mg) |
| PC | 20 | 200 | |
| PC/PG | 20 | 180 | 20 |

Table 2. Characteristics of non-coated and Carbopol-coated liposomes (n=3)

| Type of formulation | Diameter ± SD[a] (nm) | PI[b] | Zeta potential ± SD (mV) | Entrapment efficiency ± SD (%) |
|---|---|---|---|---|
| PC-non-coated | > 1µm | 0.51 | -6.05 ± 0.74 | 15.24 ± 1.85 |
| PC-coated | > 1µm | 0.56 | -10.34 ± 1.65 | 20.34 ± 5.43 |
| PC-non-coated-sonicated | 308.5 ± 28.5 (83.5%)<br>75.6 ± 12.9 (16.5%) | 0.43 | -1.71 ± 0.27 | 7.30 ± 1.63 |
| PC-coated-sonicated | 236.1 ± 21.7 (88.2%)<br>65.5 ± 11.8 (11.8%) | 0.34 | -7.65 ± 1.63 | 9.63 ± 6.39 |
| PC/PG-non-coated | 681.5 ± 41.0(90.3%)<br>152.0 ± 33.0 (9.7%) | 0.34 | -30.00 ± 2.81 | 22.12 ± 4.98 |
| PC/PG-coated | 455.3 ± 50.5 (88.4%)<br>116.1 ± 23.8 (11.6%) | 0.29 | -35.80 ± 2.68 | 17.92 ± 1.58 |
| PC/PG-non-coated-sonicated | 147.8 ± 48.2 (48.3%)<br>67.5 ± 6.0 (48.9%)<br>13.0 ± 3.4 (2.8%) | 0.27 | -23.20 ± 1.64 | 11.48 ± 7.41 |
| PC/PG-coated-sonicated | 64.4 ± 4.3 (96.2%)<br>10.1 ± 2.0 (3.8%) | 0.29 | -31.00 ± 1.48 | 5.33 ± 0.57 |

a The percentual values indicate population of liposomes present in samples

b PI (polydispersity index)