COMPOSITION AND METHOD FOR TREATING HPV

Field of the invention

The present invention relates to the treatment of viral infections. In particular, the present invention relates to formulations for treating human papillomavirus (HPV) infections and associated malignancies. The present invention specifically relates to solid dosage forms for general drug delivery, topical application or mucosal delivery.

Background

DNA-viruses such as Herpes, Pox, Papillomavirus, Adenoviruses, Smallpox viruses etc. can cause many different infectious diseases in humans. One example, Human papillomavirus (HPV) is a member of the papillomaviridae family of non-enveloped DNA viruses capable of infecting humans. Like all papillomaviruses, HPV is strictly epitheliotropic and establishes productive infections only in the stratified epithelium of the skin or mucous membranes. While the majority of the nearly 200 known types of HPV cause no symptoms in most people, some types can cause warts, while others can lead to various cancers, most notably cervical cancer.

More than 30 to 40 types of HPV are typically transmitted through sexual contact and infect the anogenital region. Some sexually transmitted HPV types may cause genital warts. Persistent infection with "high-risk" HPV types, different from the ones that cause skin warts, may progress to precancerous lesions and invasive cancer. HPV infection is a cause of nearly all cases of cervical cancer; however, most infections with these types do not cause disease.

Most HPV infections in young females are temporary and have little long-term significance. 70% of infections are gone in 1 year and 90% in 2 years. However, when infection persists (in 5% to 10% of infected women) there is high risk of developing cervical precancer (lesions on the cervix), which can progress to invasive cervical cancer. This process usually takes 15-20 years, providing many opportunities for detection and treatment of the pre-cancerous condition, often with high cure rates.

While vaccination is an effective way to prevent HPV infection, therapeutic options are limited, expensive and often not well tolerated. Classical therapeutic approaches comprise cytodestructive and cytotoxic substances, surgical methods, laser and

cryotherapy, possibly in combination with immunotherapy. In recent years, the acyclic nucleoside phosphonate cidofovir has proven to be effective in the treatment of a variety of clinical manifestations of several DNA-viruses such as Herpes, Pox, Papillomavirus, Adenoviruses, Smallpox viruses etc.. in particular, cidofovir has been used to treat HPV-induced epithelial cell proliferation. *In vitro*, treatment of HPV-positive cells with cidofovir has resulted in a concentration- and time-dependent inhibition of cell proliferation. Different parameters of apoptosis showed that the mechanism of cell death following treatment with cidofovir is based on apoptosis. Treatment with intravenous (systemic) cidofovir has been shown to result in the stabilization of disseminated papillomatosis. Local intratumor injections of cidofovir in patients with papillomatous lesions have been shown to result in a complete regression of the tumor. In addition, cidofovir topical gel has been successfully used for the treatment of severe, relapsing anogenital HPV lesions and cervical intraepithelial neoplasia. As cidofovir has been proven to be able to induce apoptosis, the regression of papillomatous tumors may be due, at least in part, to the induction of apoptosis by cidofovir.

As illustrated above, various formulations and routes of administration are presently used for the application of cidofovir. However, each application is presented with specific drawbacks. Systemic administration of an aqueous cidofovir solution, by intravenous injection, possibly leads to systemic side effects. Cidofovir concentrations need to be increased to assure adequate cidofovir amounts at the target site which may result in nephrotoxicity. On the other hand, local injections of cidofovir at the target site may require multiple injections to assure adequate coverage of the target site. As an alternative to aqueous solutions of cidofovir for injection, creams, gels and films have been developed for topical applications, which may assure localized application to the target site. However, the stability of cidofovir in creams is low and its activity therefore deteriorates fast. In addition, the preparation of films includes a heating step, which may entail a risk of heat-mediated cidofovir degradation.

It is therefore the objective of the present invention to provide novel cidofovir formulations which overcome the drawbacks of the currently known formulations.

Summary

After having performed extensive research, the inventors have found that specific formulations comprising a lyophilized composition comprising cidofovir, hydroxyethylcellulose (HEC) or hydroxypropylmethylcellulose (HPMC) and optionally a plasticizer present a valuable and advantageous alternative for the existing cidofovir formulations.

Therefore, in a first aspect, the present disclosure relates to a sheet-shaped lyophilized composition comprising:

(a) cidofovir in an amount between 0.1 and 5.0 mg/cm²;

(b) hydroxyethylcellulose (HEC) or hydroxypropylmethylcellulose (HPMC) in an amount between 1.0 and 17.0 mg/cm²; and optionally

(c) a plasticizer in an amount between 0 and 5.0 mg/cm²,

In a preferred embodiment, the lyophilized composition according to the present disclosure comprises:

(a) cidofovir in an amount between 0.1 and 5 mg/cm², preferably between 2.0 and 5.0 mg/cm²;

(b) HEC in an amount between 1.0 and 17.0 mg/cm², preferably between 7.0 and 10.5 mg/cm²; and

(c) a plasticizer in an amount between 0.5 and 4.0 mg/cm².

Preferably, the HEC is selected from the group consisting of: HEC H4000, HEC 250M, HEC 250HX and HEC 250HHX, most preferably HEC 250M or HEC 250 HX.

In other preferred embodiments, the lyophilized composition according to the present disclosure comprises:

(a) cidofovir in an amount between 0.1 and 5 mg/cm²;

(b) HPMC in an amount between 1.0 and 17.0 mg/cm²; and

(c) a plasticizer in an amount between 0.0 and 5.0 mg/cm².

Preferably, the HPMC is selected from the group consisting of HPMC E5, HPMC E15, HPMC 4000 and HPMC K15, most preferably HPMC E5, or HPMC E15.

The inventors have surprisingly found that such formulations form, after lyophilisation, a dry, solid, easy to manipulate and malleable porous matrix which presents itself as a sponge-like structure.

In contrast to the previously known creams or gels, the composition according to the present disclosure displays excellent cidofovir stability. Hence, and advantageously, the compositions according to the present disclosure can be stored at ambient (room) temperature, in contrast to gels and creams, which need to be stored refrigerated. Moreover, in contrast to the preparation of films a heating step is absent during the preparation of the compositions according to the present disclosure, thereby eliminating the risk of heat-mediated cidofovir degradation.

The addition of a small amount of water to the lyophilized composition according to the present disclosure allows for the rapid transformation of the sponge-like structure into a mucoadhesive gel without the need for agitation. It has been found that the speed of rehydration, as well as the quantity of water needed for rehydration can easily be modulated by changing the type and the concentration of the polymer. In particular, it has been found that the speed of rehydration decreases with increasing concentration of the polymer. Depending of whether a slow rehydration is needed (e.g. for slow release applications) a higher concentration of the polymer can be used in the compositions according to the present disclosure. The inventors have also found that HPMC-based sponges require a lower amount of water for rehydration than HEC-based sponges, which might influence the mode of administration. HPMC-based sponges may be administered directly, i.e. without being rehydrated before application. Rehydration which may take place *in situ*, in the uterus, with the aid of a minimal amount of vaginal fluids. This is especially true for HPMC E5, HPMC E15, HPMC 4000 and HPMC K15, most particularly for HPMC E5, and HPMC E15.

The HEC-based sponges on the other hand may be rehydrated prior to administration, resulting in a gel-like composition, which can subsequently be applied into the vagina or cervix of the subject. Rehydration of the HEC-based compositions of the present disclosure can be done with a larger quantity of water than for the HPMC-based compositions.

It has further been found that the viscosity of the gel which is obtained after rehydration of the compositions according to the present disclosure is determined by the type and the concentration of the polymer (not by the type or the concentration of the plasticizer). This allows for easy modulation of the viscosity depending on the needs by changing the type and concentration of the polymer. In particular, the inventors have found that using the same conditions and amounts of components, the HEC-based sponges are more viscous after rehydration than HPMC-based sponges and present themselves as

gels. This is especially true for HEC H4000, HEC 250M, HEC 250HX and HEC 250HHX, most particularly for HEC 250M and HEC 250 HX.

The differences in viscosity are important in determining the way of applying the composition of the present disclosure to the subject. The advantage of the HPMC-based compositions, being less viscous, is that less water is needed for the *in situ* formation of a gel-like structure. They can therefore easily be applied internally to the subject without the need for addition of water to invoke rehydration. Said rehydration will take place in situ in the body cavity of the subject, using bodily fluids. The HEC-based compositions are more viscous and have the advantage to form a gel, which can be easier applied topically to e.g. the anogenital region.

The present disclosure therefore also provides a gel-like composition obtained after rehydration of the lyophilized compositions according to the present disclosure, especially of the HEC-based lyophilized compositions according to the present disclosure.

In an embodiment, the plasticizer in the lyophilized composition according to the present disclosure, if present, is selected from the group consisting of polyethylene glycol 400 or 4000 (PEG 400 or 4000) and propylene glycol (PG). In a preferred embodiment, the plasticizer in the composition according to the present disclosure, if present, is PEG, more preferably PEG 400.

In another embodiment, the lyophilized composition according to the present disclosure further comprises water in an amount between 1 and 10 weight%, preferably between 1 and 8 weight%.

In an embodiment, the lyophilized composition according to the present disclosure further comprises NaOH. NaOH is generally added to the composition before lyophilization in order to promote the solubilization of cidofovir. In a further embodiment, the compositions according to the present disclosure have a pH between 6 and 8, preferably between 6.5 and 7.5 before lyophilization and after rehydratation of the matrix.

Preferably, the HPMC in the compositions according to the present disclosure is selected from the group consisting of HPMC E5, HPMC E15, HPMC 4000 and HPMC K15; the HEC

in the compositions according to the present disclosure is selected from the group consisting of: HEC H4000, HEC 250M, HEC 250HX and HEC 250HHX.

The inventors have shown that in contrast to lyophilized placebo compositions comprising various cellulose derivatives such as HPMC, NaCMC, or HEC, which all form malleable sponges, only HPMC- and HEC-based compositions are capable of forming malleable sponges after lyophilization when cidofovir is added.

Unexpectedly, other tested compositions, based on other polymers, such as NaCMC, Carbomer 974P or HPC in the presence of cidofovir do either not form malleable sponges, or present other unfavorable characteristics, such as being very rigid and/or brittle, being too sticky or not adherent at all, showing structural defects or show slow or difficult rehydration. The inventors have shown that the addition of cidofovir alters the structural and functional characteristics of the lyophilized sponges.Furthermore, the inventors have shown that the addition of cidofovir in the compositions according to the present disclosure alters the pore structure of the sponge, in comparison with placebo sponges. In particular, it was found that cidofovir seems to weaken the pore structure, especially in case of the HPMC based compositions.

In one embodiment, the present disclosure relates to the lyophilized composition as described herein for use in topical drug delivery especially for treating all pathologies linked to infections with human DNA-viruses such as Herpes, Pox, Papillomavirus, Adenoviruses, Smallpox viruses, preferably by HPV, CMV, BK-virus, Smallpox, HSV, or VZV, or accompanying pathologies such as those selected from the group comprising: virus induced lesions or warts of the vagina, cervix, anogenital region, mucosa, epithelium of the oral sphere, or skin, precancerous lesions and/or neoplasms or cancers caused by viral infection, more specifically to the cervix or the mucosal surface of the cervix. In particular, HPV infections and pathologies are envisaged. The term "pathologies linked to HPV or DNA-virus infections" encompasses, but is not limited to: HPV or DNA-virus induced lesions or warts of the cervix, uterus, anogenital region, or skin, precancerous lesions and/or neoplasms or cancers caused by HPV or DNA-virus infection, more specifically to the cervix or the mucosal surface of the cervix. In another embodiment, the present disclosure relates to the lyophilized composition as described herein for use in treating human papillomavirus (HPV) infection. In yet another embodiment, the present disclosure relates to the lyophilized composition as described herein for use in treating cervical cancer. The

present disclosure also provides for methods of treating HPV or DNA-virus infection, using the lyophilized compositions according to the present disclosure. Administration of said compositions can be done directly, by inserting the lyophilized composition into the cervix, e.g. on a vaginal inserter or fixed to a vaginal or uteral cap. Alternatively, the composition according to the present disclosure can be rehydrated prior to its administration and subsequently in the cervix applied as a gel-like composition. The treatment can be repeated a number of times in order to prevent, reduce or eliminate the pathologies linked to HPV or DNA-virus infections as defined herein, more in particular HPV or DNA-virus infections, or HPV or DNA-virus induced lesions, precancerous lesions, or cancers, especially cervical cancer.

In a second aspect of the present disclosure, there is provided the lyophilized composition as defined herein for use in medicine, preferably for use in medicine, preferably for use in treating infection with human DNA-virus such as Herpes virus, Pox virus, Papilloma virus, Adenovirus, Smallpox virus, or Human papillomavirus (HPV), or accompanying pathologies such as those selected from the group comprising: virus induced lesions or warts of the vagina, cervix, anogenital region, mucosa, epithelium of the oral sphere, or skin, precancerous lesions and/or neoplasms or cancers caused by HPV infection, more specifically to the cervix or the mucosal surface of the cervix.

In a third aspect of the present disclosure, there is provided a gel-like composition obtained after rehydration of the lyophilized composition as defined herein for use in medicine, preferably for use in treating infections with human DNA-viruses such as Herpes, Pox, Papillomavirus, Adenoviruses, Smallpox viruses, or Human papillomavirus (HPV) infection or accompanying pathologies such as those selected from the group comprising: virus induced lesions or warts of the vagina, cervix, anogenital region, mucosa, epithelium of the oral sphere, or skin, precancerous lesions and/or neoplasms or cancers caused by viral infection, more specifically to the cervix or the mucosal surface of the cervix.

In a fourth aspect of the present disclosure, there is provided the lyophilized composition as defined herein for use in treating human DNA virus infection or accompanying pathologies, wherein the composition is rehydrated before administration.

In a fifth aspect of the present disclosure, there is provided the lyophilized composition as defined herein, for use in treating human DNA virus infection or accompanying pathologies , wherein the composition is not rehydrated before administration.

In a sixth aspect of the present disclosure, there is provided the lyophilized composition as defined herein, or the gel-like composition as defined herein, for use in topical drug delivery to the vagina, cervix, anogenital region, mucosa, epithelium of the oral sphere, or skin.

In a seventh aspect of the present disclosure, there is provided a method for producing the lyophilized composition as defined herein, comprising the steps of (a) dispersing HEC or HPMC in water to obtain a homogenized composition; (b) optionally dispersing plasticizer in the composition obtained in step (a) to obtain a homogenized composition; (c) dispersing cidofovir and optionally adding NaOH 2M solution in the composition obtained in step (b) or alternatively the composition obtained in step (a) if no plasticizer is added to obtain a homogenized composition; (d) lyophilizing the composition obtained in step (c), wherein plasticizer needs to be added in step (b) when HEC is used in step (a).

In a further embodiment, the present disclosure relates to methods for producing the lyophilized composition as described herein, comprising the steps of:

(a) dispersing a polymer in water to obtain a homogenized composition;

(b) optionally dispersing a plasticizer in the composition obtained in step (a) to obtain a homogenized composition;

(c) dispersing cidofovir and optionally adding NaOH 2M solution in the composition obtained in step (b) or alternatively the composition obtained in step (a) if no plasticizer is added to obtain a homogenized composition;

(d) lyophilizing the composition obtained in step (c).

In a preferred embodiment of the method of the present disclosure,

(a) cidofovir is added in an amount between 0.1 and 5.0 mg/cm^2;

(b) hydroxyethylcellulose (HEC) or hydroxypropylmethylcellulose (HPMC) is added in an amount between 1.0 and 17.0 mg/cm^2; and optionally

(c) a plasticizer is added in an amount between 0 and 5.0 mg/cm^2,

wherein between 0.5 and 4.0 mg/cm^2 of said plasticizer is present when HEC is used.

In a particularly preferred embodiment of the method according to the present disclosure,

(a) cidofovir is added in an amount between 0.1 and 5 mg/cm^2, preferably between 2.0 and 5.0 mg/cm^2;

(b) HEC is added in an amount between 1.0 and 17.0 mg/cm^2, preferably between 7.0 and 10.5 mg/cm^2; and

(c) a plasticizer is added in an amount between 0.5 and 4.0 mg/cm^2.

Preferably, the HEC in the compositions according to the present disclosure is selected from the group consisting of: HEC H4000, HEC 250M, HEC 250HX and HEC 250HHX, most preferably HEC 250M and 250HX.

In a further embodiment, the homogenized composition obtained in step (c) comprises NaOH in an amount between 0.10 to 0.30 weight%, preferably about 0.20 or 0.21 %, before lyophilisation.

In other preferred embodiments, the lyophilized composition according to the present disclosure comprises:

(a) cidofovir is added in an amount between 0.1 and 5 mg/cm^2;

(b) HPMC is added in an amount between 1.0 and 17.0 mg/cm^2; and

(c) a plasticizer is added in an amount between 0.0 and 5.0 mg/cm^2.

Preferably, said HPMC is selected from the group consisting of HPMC E5, HPMC E15, HPMC 4000 and HPMC K15, most preferably HPMC E5 and E15.

In a further embodiment, the homogenized composition obtained in step (c) comprises NaOH in an amount between 0.10 to 0.30 weight%, preferably about 0.20 or 0.21 %, before lyophilisation.

Preferably, said plasticizer is selected from the group consisting of polyethylene glycol 400 or 4000 (PEG 400 or 4000) and propylene glycol (PG). In a preferred embodiment, the plasticizer in the composition according to the present disclosure, if present, is PEG, more preferably PEG 400.

In a further embodiment, the homogenized composition obtained in step (c) comprises between 0.35 and 3.5 weight% of HEC or HPMC. In particular embodiments, the homogenized composition obtained in step (c) comprises between 1 and 2.5 weight% HEC and between 0.2 and 1 weight% plasticizer. In other particular embodiments the homogenized composition obtained in step (c) comprises between 0.35 and 3.5 weight% HPMC and between 0 and 1 weight% plasticizer. In yet another embodiment, the homogenized composition of step (c) comprises between 0.10 and 1.5 weight% cidofovir. In an embodiment, the homogenized composition obtained in step (c) has a pH between 6 and 8, preferably between 6.5 and 7.5.

In a further embodiment, the homogenized composition obtained in step (c) comprises NaOH in an amount between 0.10 to 0.30 weight%, preferably about 0.20 or 0.21 %, before lyophilisation.

In an embodiment, the lyophilization for producing the compositions according to the present disclosure can be performed in a crystallizer, or in a mould, or in any other known device or reactor that can be used to make a sheet-like lyophilized composition of the present disclosure.

In an eighth aspect of the present disclosure, there is provided a method of preparing a gel-like composition as defined herein, comprising the step of rehydrating the lyophilized composition as defined herein.

In a ninth aspect of the present disclosure, there is provided a sheet-shaped solid porous malleable matrix obtained by lyophilization of an aqueous composition, said aqueous composition comprising between 1 and 17 mg/cm² HEC or HPMC, between 0 and 5 mg/cm² plasticizer, and between 0.1 and 5 mg/cm² cidofovir, wherein between 1.5 and 3 mg/cm² of said plasticizer is present when HEC is used.

Another embodiment of the present disclosure relates to a sheet-shaped solid porous malleable matrix obtained by lyophilization of an aqueous composition, said aqueous composition comprising between 0.35 and 3.5 weight% HEC or HPMC, between 0 and 1 weight% plasticizer, and between 0.10 and 1.5 weight% cidofovir.

In particular embodiments this matrix comprises between 1 and 2.50 weight% HEC, between 0.2 and 1 weight% plasticizer, and between 0.10 and 1.5 weight% cidofovir.

In other particular embodiments this matrix comprises between 0.35 and 3.50 weight% HPMC, between 0 and 1 weight% plasticizer, and between 0.10 and 1.5 weight% cidofovir.

In an embodiment, this matrix comprises between 1 and 17 mg/cm² HEC or HPMC, between 0 and 5 mg/cm² plasticizer, and between 0.1 and 5 mg/cm² cidofovir.

In particular embodiments, this matrix comprises between 7 and 10.5 mg/cm² HEC, between 1.5 and 5 mg/cm² plasticizer, and between 0.1 and 5 mg/cm² cidofovir, preferably with HEC 250M or HEC 250HX as the polymer.

In other particular embodiments, this matrix comprises between 1 and 17 mg/cm² HPMC, between 0 and 5 mg/cm² plasticizer, and between 0.1 and 5 mg/cm² cidofovir, preferably with HPMC E5 or E15 as the polymer.

In a tenth aspect of the present disclosure, there is provided a drug delivery applicator, comprising the sheet-shaped lyophilized composition as defined herein or the matrix as defined herein.

A further embodiment of the present disclosure relates to a drug delivery applicator, comprising the sheet-shaped lyophilized composition or the malleable matrix according to the present disclosure as described herein disposed on a drug-impermeable barrier.

In an embodiment, the drug-impermeable barrier is a cap that is configured to fit over an outer periphery of a cervix.

Brief description of the figures

Figure 1: HPLC chromatogram of cidofovir showing absorbance (AU) in function of retention time (min). (A) chromatogram of calibrated cidofovir; (B) chromatogram of cidofovir from a lyophilized composition comprising HPMC E5 according to an embodiment of the invention at T0; (C, D, E, F and G) chromatogram of cidofovir from a lyophilized composition according to an embodiment of the invention respectively at T1, T3, T6, T9 and T12, i.e. after 1, 3, 6, 9 and 12 month(s) of storage at 45 °C.

Figure 2: HPLC chromatogram of cidofovir showing absorbance (AU) in function of retention time (min). (A) chromatogram of cidofovir from a lyophilized composition comprising HEC 250HX according to an embodiment of the invention at T0; (B and C) chromatogram of cidofovir from a lyophilized composition according to an embodiment of the invention at T1 and T3, i.e. after 1 and 3 months of storage at 45°C.

Figure 3: Diffusion kinetics of cidofovir, as measured with a Franz diffusion cell, showing the percentage (%) of diffused cidofovir in function of time (hours). (A) diffusion kinetics of cidofovir in various lyophilized compositions according to an embodiment of the invention (HPMC E5-based) in comparison to carbomer gel (prior art); (B) diffusion kinetics of cidofovir in various NaCMC-based compositions in comparison to carbomer gel (prior art); (C) diffusion kinetics of cidofovir in various HEC 250M and 250HX-based compositions in comparison to carbomer gel (prior art).

Figure 4: Scanning electron microscopy (SEM) at 25x magnification of the external layer of compositions comprising HPMC E5 according to an embodiment of the invention. (left image) SEM of a composition according to an embodiment of the invention comprising PEG 400 as a plasticizer; (right image) SEM of a composition according to an embodiment of the invention comprising PG as a plasticizer.

Figure 5: Scanning electron microscopy (SEM) at 100x magnification of compositions comprising HPMC E5. (A) SEM of a placebo composition comprising PEG 400 (left image) or PG (right image) as a plasticizer; (B) SEM of a composition according to an

embodiment of the invention comprising PEG 400 (left image) or PG (right image) as a plasticizer.

Figure 6: Scanning electron microscopy (SEM) at 4000x magnification of compositions comprising HPMC E5. (A) SEM of a placebo composition (left image) or a composition according to an embodiment of the invention (right image) each comprising PEG 400 as a plasticizer; (B) SEM of a placebo composition (left image) or a composition according to an embodiment of the invention (right image) each comprising PG as a plasticizer.

Figure 7: Scanning electron microscopy (SEM) of placebo compositions comprising HPMC E5 as polymer and PEG 400 as plasticizer. (A) SEM at 25x magnification of the external layer of a composition comprising 7,95 mg/cm² (left image) or 15,91 mg/cm² (right image) of polymer. (B) SEM at 100x magnification of a composition comprising 7,95 mg/cm² (left image) or 15,91 mg/cm² (right image) of polymer.

Figure 8: Scanning electron microscopy (SEM) of placebo compositions comprising HPMC E5. (A) SEM at 25x magnification of the external layer of a composition comprising 0 mg/cm² (left image) or 1,98 mg/cm² (middle image) or 3,97 mg/cm² (right image) of PEG 400 as plasticizer. (B) SEM at 100x magnification of a composition comprising 0 mg/cm² (left image) or 1,98 mg/cm² (middle image) or 3,97 mg/cm² (right image) of PEG 400 as plasticizer.

Figure 9: Scanning electron microscopy (SEM) at 100x magnification of a placebo composition (A) or a compositon according to an embodiment of the invention (B) comprising HPMC E5 (left image) or HPMC 4000 (middle image) or HPMC K15 (right image) as polymer.

Figure 10: Scanning electron microscopy (SEM) of compositions comprising HEC 250HX. (A) SEM at 25x magnification of the external layer of a placebo composition comprising 1,98 mg/cm² of PEG 400 as plasticizer (left image) or a compositon according to an embodiment of the invention comprising 1.98 mg/cm² of PEG 400 as plasticizer (right image). (B) SEM at 100x magnification of a placebo composition comprising 1,98 mg/cm² of PEG 400 as plasticizer (left image) or a compositon

according to an embodiment of the invention comprising 1.98 mg/cm² of PEG 400 as plasticizer (right image).

Figure 11: Scanning electron microscopy (SEM) of compositions comprising HEC 250HX as polymer. SEM of a placebo composition at 4000x magnification (left image) or a composition according to an embodiment of the invention at 750x magnification (middle image) or a composition according to an embodiment of the invention at 4000x magnification (right image) each comprising PEG 400 as a plasticizer.

Figure 12: Viscosity of rehydrated lyophilized compositions comprising NaCMC or compositions comprising HPMC according to an embodiment of the invention. Viscosity (Pa.s) is shown in function of rotation speed (s^{-1}). (top graph) general overview; (bottom graph) detail of the indicated portion in the top graph. The order of the references follows the order of the curve in the figure, starting from the top curve.

Figure 13: Viscosity of rehydrated lyophilized compositions comprising NaCMC or compositions comprising HPMC E5 according to an embodiment of the invention in function of the plasticizer. Viscosity (Pa.s) is shown in function of rotation speed (s^{-1}). The order of the references follows the order of the curve in the figure, starting from the top curve.

Figure 14: Comparison of the viscosity of a gel comprising HEC 250M 4% (without lyophilization), a sponge comprising HEC 250M 4% after rehydratation with water ad 3g and a carbomer gel with cidofovir. Viscosity (Pa.s) is shown in function of rotation speed (s^{-1}). (top graph) general overview; (bottom graph) detail of the indicated portion in the top graph. The order of the references follows the order of the curve in the figure, starting from the top curve.

Figure 15: Comparison of the viscosity of a gel comprising HEC 250HX 3% (without lyophilization), a sponge comprising HEC 250HX 3% after rehydratation with water ad 3g and a carbomer gel with cidofovir. Viscosity (Pa.s) is shown in function of rotation speed (s^{-1}). (top graph) general overview; (bottom graph) detail of the indicated portion in the top graph. The order of the references follows the order of the curves in the figure, starting from the top curve.

Figure 16: Viscosity of rehydrated lyophilized compositions comprising different types of HPMC or HEC according to an embodiment of the invention. Compositions are classified in function of their viscosity. Viscosity (Pa.s) is shown in function of rotation speed (s^{-1}). (top graph) general overview; (bottom graph) detail of the indicated portion in the top graph. The order of the references follows the order of the curves in the figure, starting from the top curve.

Figure 17: Differential scanning calorimetry of dehydrated cidofovir in powder form in comparison with compositions according to an embodiment of the invention. Heat flow (mW) is shown in function of time (min) and temperature (°C).

Detailed description

Before the present method and products of the invention are described, it is to be understood that this invention is not limited to particular methods, components, products or combinations described, as such methods, components, products and combinations may, of course, vary. It is also to be understood that the terminology used herein is not intended to be limiting.

As used herein, the singular forms "a", "an", and "the" include both singular and plural referents unless the context clearly dictates otherwise.

The terms "comprising", "comprises" and "comprised of" as used herein are synonymous with "including", "includes" or "containing", "contains", and are inclusive or open-ended and do not exclude additional, non-recited members, elements or method steps. It will be appreciated that the terms "comprising", "comprises" and "comprised of" as used herein comprise the terms "consisting of", "consists" and "consists of".

The recitation of numerical ranges by endpoints includes all numbers and fractions subsumed within the respective ranges, as well as the recited endpoints.

The term "about" or "approximately" as used herein when referring to a measurable value such as a parameter, an amount, a temporal duration, and the like, is meant to encompass variations of +/-10% or less, preferably +/-5% or less, more preferably +/-1% or less, and still more preferably +/-0.1% or less of and from the specified value,

insofar such variations are appropriate to perform in the disclosed invention. It is to be understood that the value to which the modifier "about" or "approximately" refers is itself also specifically, and preferably, disclosed.

Whereas the terms "one or more" or "at least one", such as one or more or at least one member(s) of a group of members, is clear per se, by means of further exemplification, the term encompasses inter alia a reference to any one of said members, or to any two or more of said members, such as, e.g., any ≥3, ≥4, ≥5, ≥6 or ≥7 etc. of said members, and up to all said members.

All references cited in the present specification are hereby incorporated by reference in their entirety. In particular, the teachings of all references herein specifically referred to are incorporated by reference.

Unless otherwise defined, all terms used in disclosing the invention, including technical and scientific terms, have the meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. By means of further guidance, term definitions are included to better appreciate the teaching of the present invention.

In the following passages, different aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances of the phrases "in one embodiment" or "in an embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment, but may. Furthermore, the particular features, structures or characteristics may be combined in any suitable manner, as would be apparent to a person skilled in the art from this disclosure, in one or more embodiments. Furthermore, while some embodiments described herein include some but not other features included in other embodiments, combinations of features of different embodiments are

meant to be within the scope of the invention, and form different embodiments, as would be understood by those in the art. For example, in the appended claims, any of the claimed embodiments can be used in any combination.

The invention relates to a sheet-shaped lyophilized composition comprising
(a) cidofovir in an amount between 0.1 and 5 mg/cm²;
(b) hydroxyethylcellulose (HEC) or hydroxypropylmethylcellulose (HPMC) in an amount between 1.5 and 17 mg/cm²; and optionally
(c) a biocompatible plasticizer in an amount between 0 and 5 mg/cm².

Particularly preferred embodiments of compositions according to the invention are listed in Tables A to D. Tables A-D list the concentrations of the excipients after lyophilization in mg/cm² and before lyophilization in weight% (with HPMC as polymer and without plasticizer in Table A; with HPMC as polymer and with PEG 400 as plasticizer in Table B; with HPMC as polymer and with PG as plasticizer in Table C; with HEC as polymer and PEG 400 as plasticizer in Table D). Values in Tables A to D are indicated with a deviation of ±10% of each specific value (e.g. 3.97 mg/cm² HPMC E5 ±10% ranges between 3.97-0.397 mg/cm² and 3.97+0.397 mg/cm²; 0.42% PEG 400 ±10% ranges between 0.42-0.042% and 0.42+0.042%).

Table A

HPMC			cidofovir		total weight
Type	mg/cm²	%	mg/cm²	%	g
E5	3.97±10%	0.83±10%	4.77±10%	1±10%	6
E5	7.95±10%	1.43±10%	4.77±10%	0.86±10%	7
E5	7.95±10%	1.67±10%	4.77±10%	1±10%	6

Table B

HPMC			PEG 400		cidofovir		total weight
Type	mg/cm²	%	mg/cm²	%	mg/cm²	%	g
E5	2.45±10%	1±10%	0.49±10%	0.2±10%	0.36±10%	0.15±10%	10
E5	3.14±10%	1±10%	0.63±10%	0.2±10%	0.94±10%	0.3±10%	5
E5	3.97±10%	0.83±10%	1.98±10%	0.42±10%	4.77±10%	1±10%	6
E5	6.36±10%	1.33±10%	1.98±10%	0.42±10%	4.77±10%	1±10%	6
E5	7.95±10%	1.43±10%	1.98±10%	0.36±10%	4.77±10%	0.86±10%	7
E5	7.95±10%	1.67±10%	1.98±10%	0.42±10%	4.77±10%	1±10%	6

E5	7.95±10%	1.67±10%	1.98±10%	0.42±10%	2.38±10%	0.5±10%	6
E5	11.93±10%	2.5±10%	1.98±10%	0.42±10%	4.77±10%	1±10%	6
E5	12.57±10%	1.67±10%	2.51±10%	0.33±10%	1.88±10%	0.25±10%	12
E5	15.91±10%	3.33±10%	1.98±10%	0.42±10%	2.387±10%	0.5±10%	6
E5	3.97±10%	0.83±10%	3.97±10%	0.83±10%	4.77±10%	1±10%	6
E5	6.366±10%	1.33±10%	3.97±10%	0.83±10%	4.77±10%	1±10%	6
E5	7.95±10%	1.67±10%	3.97±10%	0.83±10%	4.77±10%	1±10%	6
E5	7.95±10%	1.67±10%	3.97±10%	0.83±10%	2.38±10%	0.5±10%	6
E5	15.91±10%	3.33±10%	3.97±10%	0.83±10%	4.77±10%	1±10%	6
E15	7.95±10%	1.67±10%	1.98±10%	0.42±10%	4.77±10%	1±10%	6
4000	7.95±10%	1.67±10%	1.98±10%	0.42±10%	4.77±10%	1±10%	6
4000	9.54±10%	2±10%	1.98±10%	0.42±10%	4.77±10%	1±10%	6
4000	9.54±10%	2±10%	3.97±10%	0.83±10%	4.77±10%	1±10%	6
K15	7.95±10%	1.67±10%	1.98±10%	0.42±10%	4.77±10%	1±10%	6

Table C

HPMC			PG		cidofovir		total weight
Type	mg/cm²	%	mg/cm²	%	mg/cm²	%	g
E5	7.95±10%	1.67±10%	1.98±10%	0.42±10%	2.38±10%	0.5±10%	6
4000	1.98±10%	0.42±10%	3.97±10%	0.83±10%	2.38±10%	0.5±10%	6
K15	1.98±10%	0.42±10%	3.97±10%	0.83±10%	2.38±10%	0.5±10%	6

Table D

HEC			PEG 400		cidofovir		total weight
Type	mg/cm²	%	mg/cm²	%	mg/cm²	%	g
250 M	7.95±10%	1.67±10%	1.98±10%	0.42±10%	2.38±10%	0.5±10%	6
250 M	9.54±10%	2±10%	1.98±10%	0.42±10%	4.77±10%	1±10%	6
250 M	9.54±10%	2±10%	3.58±10%	0.75±10%	4.77±10%	1±10%	6
250 HX	7.95±10%	1.67±10%	1.98±10%	0.42±10%	4.77±10%	1±10%	6

In another embodiment, the invention provides a pharmaceutical composition comprising a therapeutically effective amount of the lyophilised composition described above and a further pharmaceutically acceptable carrier.

In an aspect of the embodiment, the pharmaceutical composition further comprises an anti-viral agent. The anti-viral agent can be an Interferon, imiquimod, formaldehyde, glutaral, cimetidine, 5-fluorouracil, trichloroacetic acid, bleomycin, podofilox, podophyllum or any other anti-viral composition usefull for the treatment of HPV infections, HPV infected tissue, or HPV infected cells.

In still another embodiment, the invention provides a method of treating HPV infections, HPV infected tissue or HPV infected cells comprising contacting the infectious site, infected tissue or cells with the lyophilized composition described herein. In an aspect of the invention, the method further comprises contacting the cells with an anti-viral agent. The anti-viral agent can be an Interferon, imiquimod, formaldehyde, glutaral, cimetidine, 5-fluorouracil, trichloroacetic acid, bleomycin, podofilox, podophyllum, or any other anti-viral composition usefull for the treatment of HPV infections, HPV infected tissue, or HPV infected cells. Said further anti-viral agent can be comprised in the lyophilized composition of the invention, or can be administered simultaneously, prior to, or following the administration of the lyophilized composition according to the present invention.

More than 150 types of HPV are acknowledged to exist. Of these, the following have been classified as types involving risk for cervical cancer: HPV-1, 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 66, 68, 70, 72, 73, 81, 82, and CP6108. Types 16 and 18 are generally acknowledged to cause about 70% of cervical cancer cases. Together with type 31, they are the prime risk factors for cervical cancer. In any of the embodiments described herein, said HPV can be HPV 11, HPV16, HPV18, HPV1, HPV6, any further type of HPV listed above, and any combination thereof.

Administration of the lyophilized composition according to the present invention, or the pharmaceutical composition as defined herein can be done directly, i.e. without rehydration of the lyophilized matrix, by using e.g. a vaginal inserter, a tampon inserter, a cervical cap, a cervix-covering pessary, or any other tool that can be used to position the lyophilized matrix at the HPV infected tissue or cells. In this scenario, the lyophilized structure will be rehydrated *in situ*, using bodily fluids naturally present at the site of application.

Alternatively, the lyophilized composition according to the present invention, or the pharmaceutical composition as defined herein can be rehydrated prior to use, creating a gel-like composition which can be topically applied by using e.g. a vaginal cream inserter, a tampon inserter, a cervical cap, a cervix-covering pessary or any other tool that can be used to position the gel at the HPV infected tissue or cells.

According to the invention an "effective amount" of the composition or pharmaceutical composition is that amount effective for treating or lessening the severity of HPV infections.

Typically, HPV infections occur at the skin, mucosa, anogenital region, vulva, vagina, and the cervix (the passage between the vagina and the uterus), in which they may cause lesions, precancerous lesions, genital warts, polyps, cysts, benign neoplasms and eventually cancers, that can metastasis into the underlying tissues and circulate into the blood vessels. Cervical cancer may present with vaginal bleeding, but symptoms may be absent until the cancer is in its advanced stages. In addition, epithelial HPV infections of the oral cavity or sphere are frequently occurring. The oral cavity or sphere encompasses the lips, mouth, throat, larynx, etc. The preferred target sites for the (pharmaceutical) composition according to the present invention is therefore the vulvar, vaginal, and cervical region, especially the mucosal tissue in these areas, which are most often infected with HPV.

The invention will now be illustrated by means of the following examples, which do not limit the scope of the invention in any way.

Examples

Example 1: lyophilization conditions

Lyophilization of aqueous compositions comprising a bioadhesive polymer, and optionally a plasticizer and/or cidofovir to obtain a porous malleable matrix was performed according to the following protocol.

The polymer is dispersed in distilled water under slow agitation until complete homogenization. Optionally, the obtained dispersion is agitated again until complete homogenization after the addition of the plasticizer. Cidofovir is dispersed and a 2M NaOH stock solution is added to reach pH 7. The obtained mixture is transferred to a crystallizer and is lyophilized. The lyophilization conditions are the following:

Freezing

Stage	Temperature (°C)	Time (h)	Pressure (bar)
1	- 35 (-30 to -35)	3.0 (2 to 4)	ambient
2	- 35 (-30 to -35)	0.5 (0.4 to 0.6)	ambient

Primary drying

Stage	Temperature (°C)	Time (h)	Pressure (bar)
1	- 15 (-10 to -20)	3.0 (2 to 4)	0.8 (0.7 to 0.9)
2	- 10 (-10 to -20)	12.0 (7 to 17)	0.1 (0.05 to 0.15)

Secondary drying

Stage	Temperature (°C)	Time (h)	Pressure (bar)
1	10 (5 to 15)	2.0 (1 to 3)	0.1 (0.05 to 0.15)
2	10 (5 to 15)	3.0 (2 to 4)	0.1 (0.05 to 0.15)

Example 2: components of the lyophilized composition

The following components and mixtures were evaluated for their capacity to result in the desired "sponge" structure after lyophilization.

Bioadhesive polymers:

- Hydroxypropylmethylcellulose (HPMC)
  - HPMC E5 : viscosity: 5 mPa.s (= 5 cp) (aqueous solution of 2%)
  - HPMC E15 : viscosity: 12-18 mPa.s (aqueous solution of 2%)
  - HPMC 4000 : viscosity: 4000-5600 mPa.s (aqueous solution of 2%)
  - HPMC K15 : viscosity: 11250-21000 mPa.s (aqueous solution of 2%)
- Sodium carboxymethyl cellulose (NaCMC)
- Hydroxyethylcellulose (HEC)
  - HEC Natrosol 250HX: viscosity : 1500-2500 mPa.s (aqueous solution of 1%)
  - HEC Natrosol 250HHX: viscosity : 3500-5500 mPa.s (aqueous solution of 1%)
  - HEC Natrosol 250M: viscosity : 4500-6500 mPa.s (aqueous solution of 2%)
  - HEC H4000: viscosity : 4500-6500 mPa.s (aqueous solution of 2%)
- Carbomer 974P
- Hydroxyproprylcellulose (HPC)
  - HPC LF: viscosity : 75-150 mPa.s (aqueous solution of 5%)
  - HPC HF: viscosity : 1500-3000 mPa.s (aqueous solution of 1%)

HPC GF: viscosity : 150-400 mPa.s (aqueous solution of 2%)

Plasticizers:

- polyethylene glycol 400 (PEG 400)
- polyethylene glycol 4000 (PEG 4000)
- propylene glycol (PG)

Example 3: evaluation of different lyophilized placebo compositions

Different conditions and concentrations of the components were tested to evaluate the desired characteristics of the lyophilisate. Desired characteristics are a sponge texture which is easily malleable when dry and which can be easily and/or rapidly rehydrated into a gel with intermediate viscosity (i.e. not too liquid and not too viscous).

The following conditions were kept constant for ease of comparison: the diameter of the crystallizer (4 cm), the quantity of water used for the dispersion of the components (ad 6 g, meaning water was added to the composition up to 6g of the final composition before lyophilisation), and the lyophilization cycle.

In first instance, placebo lyophilisates were evaluated (i.e. without cidofovir). Table1 lists the tested concentrations of polymer and plasticizer.

Table 1

Polymer	mg/cm²	Plasticizer	mg/cm²	remarks
HPMC E5	3.97	-	-	malleable sponge
HPMC E5	7.95	-	-	malleable sponge
HPMC 4000	9.54	-	-	malleable sponge
HPMC 4000	11.93	-	-	malleable sponge
HPMC E5	3.97	PEG 400	1.98	malleable sponge
HPMC E5	6.36	PEG 400	1.98	malleable sponge
HPMC E5	7.95	PEG 400	1.98	malleable sponge
HPMC E5	11.93	PEG 400	1.98	malleable sponge
HPMC E5	15.91	PEG 400	1.98	malleable sponge
HPMC E5	3.97	PEG 400	3.97	malleable sponge
HPMC E5	6.36	PEG 400	3.97	malleable sponge
HPMC E5	7.95	PEG 400	3.97	malleable sponge
HPMC E5	15.91	PEG 400	3.97	malleable sponge
HPMC E5	31,83	PEG 400	7,95	malleable sponge
HPMC E5	71,62	PEG 400	7,95	malleable sponge
HPMC E15	6.36	PEG 400	1.98	malleable sponge

HPMC E15	7.95	PEG 400	1.98	malleable sponge
HPMC 4000	3.97	PEG 400	1.98	malleable sponge
HPMC 4000	6.36	PEG 400	1.98	malleable sponge
HPMC 4000	7.95	PEG 400	1.98	malleable sponge
HPMC 4000	9.54	PEG 400	1.98	malleable sponge
HPMC 4000	11.93	PEG 400	1.98	malleable sponge
HPMC K15	1.98	PEG 400	0.99	malleable sponge
HPMC K15	7.95	PEG 400	1.98	malleable sponge
HPMC E5	7.95	PEG 4000	0.79	malleable sponge
HPMC E5	7.95	PEG 4000	1.19	malleable sponge
HPMC E5	7.95	PEG 4000	1.98	malleable sponge
HPMC E5	7.95	PG	1.98	malleable sponge
HPMC E5	7.95	PG	3.97	malleable sponge
HPMC E15	6.36	PG	3.97	malleable sponge
HPMC E15	7.95	PG	1.98	malleable sponge
HPMC E15	11.93	PG	3.97	malleable sponge
HPMC 4000	1.98	PG	1.98	malleable sponge
HPMC 4000	3.97	PG	1.98	malleable sponge
HPMC K15	1.98	PG	1.98	malleable sponge
HPMC K15	3.97	PG	1.98	malleable sponge
NaCMC	3.18	PEG 400	1.98	malleable sponge
NaCMC	4.77	PEG 400	1.98	malleable sponge
NaCMC	5.57	PEG 400	1.98	malleable sponge
NaCMC	6.36	PEG 400	1.98	malleable sponge
NaCMC	4.77	PEG 400	3.97	malleable sponge
NaCMC	9.55	PEG 400	3.97	malleable sponge
NaCMC	4.77	PG	1.98	malleable sponge
NaCMC	4.77	PG	3.97	malleable sponge
HEC H4000	9.54	-	-	malleable sponge
HEC H4000	11.93	-	-	malleable sponge
HEC H4000	9.54	PEG 400	1.98	malleable sponge
HEC H4000	11.93	PEG 400	1.98	malleable sponge
HEC H4000	11.93	PEG 400	3.97	malleable sponge
HEC 250 M	9.54	-	-	malleable sponge
HEC 250 M	11.93	-	-	malleable sponge
HEC 250 M	3.97	PEG 400	1.98	malleable sponge
HEC 250 M	4.77	PEG 400	1.98	malleable sponge
HEC 250 M	7.95	PEG 400	1.98	malleable sponge
HEC 250 M	9.54	PEG 400	1.98	malleable sponge

HEC 250 M	11.93	PEG 400	1.98	malleable sponge
HEC 250 M	11.93	PEG 400	3.97	malleable sponge
HEC 250 HX	7.16	-	-	malleable sponge
HEC 250 HX	7.95	-	-	malleable sponge
HEC 250 HX	11.93	-	-	malleable sponge
HEC 250 HX	3.97	PEG 400	1.98	malleable sponge
HEC 250 HX	7.95	PEG 400	1.98	malleable sponge
HEC 250 HX	11.93	PEG 400	1.98	malleable sponge
HEC 250 HHX	4.77	-	-	malleable sponge
HEC 250 HHX	11.93	-	-	malleable sponge
HEC 250 HHX	4.77	PEG 400	1.98	malleable sponge
HEC 250 HHX	11.93	PEG 400	1.98	malleable sponge
HEC 250 HHX	11.93	PEG 400	3.97	malleable sponge
Carbomer	3.77	PEG 400	1.25	powder
Carbomer	1.47	PEG 400	0.98	powder
Carbomer	1.93	PEG 400	0.99	powder
Carbomer	1.79	PEG 400	0.99	powder

HPC GF	19.09	-	-	malleable sponge
HPC GF	23.87	-	-	malleable sponge
HPC GF	19.09	PEG 400	4.77	no sponge texture
HPC GF	23.87	PEG 400	5.96	film
HPC GF	23.87	PEG 400	7.95	film
HPC LF	7.95	PEG 400	1.98	sticky sponge
HPC LF	11.93	PEG 400	1.98	sticky sponge
HPC HF	1.98	PEG 400	0.99	sticky sponge

HPMC E5 HPC GF	7,95 1,98	PEG 400	3,97	malleable sponge
HPMC E5 HPC GF	7,95 3,97	PEG 400	3,97	malleable sponge

HPMC 4000 HPC GF	3,97 1,98	PEG 400	3,97	malleable sponge
HPMC 4000 HPC GF	3,97 3,97	PEG 400	3,97	malleable sponge
HPMC 4000 HPC GF	6,36 1,98	PEG 400	3,97	malleable sponge
HPMC 4000 HPC GF	6,36 3,97	PEG 400	3,97	malleable sponge
HPMC 4000 HPC GF	7,95 1,98	PEG 400	3,97	malleable sponge

HPMC 4000 HPC GF	7,95 3,97	PEG 400	3,97	malleable sponge

HPMC 4000 HPC GF	6,36 3,97	PEG 4000	1,19	malleable sponge
HPMC 4000 HPC GF	7,95 3,97	PEG 4000	1,19	malleable sponge

It was found that compositions based on HPMC, HEC and NaCMC allowed for the formation of sponges with the desired characteristics, irrespective of the tested concentrations and the viscosity of the polymer. Compositions based on HPMC E5, HPMC E15, HPMC 4000, as well as HPMC K15 and compositions based on HEC H4000, HEC 250 M, HEC 250 HHX, as well as HEC 250 HX allowed for the formation of sponges. Compositions based on HPC GF allowed for the formation of sponges with the desired characteristics when used in the absence of a plasticizer, while the presence of a plasticizer resulted in the formation of bioadhesive films or did not show a sponge-like texture. The compositions based on carbomer and HPC LF and HPC HF resulted respectively in powder or in sponges which were very sticky and not very malleable. Sponges with the desired characteristics were also obtained with compositions based on a combination of HPMC E5 and HPC GF and a combination of HPMC 4000 and HPC GF, all in the presence of plasticizer.

The sponges based on PEG seemed to be more resistant to rupture than the sponges based on PG. Both, PEG 400 and PEG 4000 may be used to obtain sponges with the desired characteristics, although higher concentrations of PEG 4000 often result in britlle sponges.

Residual amounts of water for the different types of polymers are listed in Table 2.

Table 2

HPMC	1.5 – 8 weight%
NaCMC	5.5 – 14 weight%
HEC	1-6 weight%
HPC	3.5-7 weight%
Carbomer	not recoverable for residual water determination

NaCMC-based sponges appear to retain more residual water than for instance HPMC-based sponges. It has been found that varying the amount of water for preparing the

dispersion (4, 5 and 6 ml) did not influence the final residual amount of water after lyophilization.

For the evaluation of the speed of rehydration, discs of 1.2 cm diameter were rehydrated with 50 µl distilled water or the entire sponge ad 3 g with distilled water. Rehydration of the sponge resulted in the formation of a gel with varying viscosity. The speed of rehydration depended on the nature and the quantity of the polymer, and hence can easily be modulated. For instance, it has been found that HEC 250HX- and 250M-based sponges allowed for a faster rehydration than HEC 250HHX- and H4000-based sponges. Irrespective of the polymer, the speed of rehydration diminished with an increasing concentration of polymer.

Example 4: evaluation of different lyophilized cidofovir-containing compositions

Different conditions and concentrations of the components were tested to evaluate the desired characteristics of the lyophilisate. Desired characteristics are a sponge texture which is easily malleable when dry and which can be rapidly rehydrated into a gel with intermediate viscosity (i.e. not too liquid and not too viscous).

The lyophilization cycle was kept constant for ease of comparison, and in most conditions the diameter of the crystallizer was 4 cm and the quantity of water added for the dispersion of the components was ad 6 g.

Tables 3 to 45 list the conditions and evaluation for each of the tested compositions. Indicated is the concentration in mg/cm² lyophilized sponge and the weight% in the aqueous composition before lyophilization of the type of polymer (HPMC, NaCMC, HEC, Carbomer or HPC) and the type of plasticizer (no plasticizer, PEG 400 or PG) and cidofovir. Additionally indicated is the amount of NaOH (provided as a 2M stock solution), the diameter of the crystallizer, and hence the sponge after lyophilization (circular structure), the weight of the aqueous composition before lyophilization, the weight of the sponge after lyophilization and the percentage of residual water in the sponge after lyophilization.

It can be seen that the addition of cidofovir to the composition modifies the properties of the sponge. Whereas placebo sponges with good quality could be obtained based

on HPMC, HEC and NaCMC as a polymer, it appears that NaCMC-based sponges with cidofovir become very rigid and brittle (irrespective of concentrations and conditions). HPMC-based sponges are malleable and of good quality, although they have a slightly different appearance than their placebo counterparts and present very faint cracks when the sponge is folded in two. In contrast to their placebo counterparts, HEC 250HX-based sponges that contain cidofovir need a plasticizer to avoid rupture of the sponges (too brittle).

It appears that PG based sponges are only slightly malleable and brittle, in contrast to PEG 400 based sponges, which are malleable and of good quality.

It has been found that irrespective of the tested polymer, the speed of rehydration decreases when the concentration of the polymer increases.

Tables 3 to 45 show some specific examples of compositions that present good-quality malleable sponges after lyophilisation. Their rehydration capacities have also been tested in some cases. The conditions in which non-malleable sponges were obtained are also shown for comparison.

Table 3

HPMC E5	mg/cm²	7.95
	%	1.43
Cidofovir	mg/cm²	4.77
	%	0.86
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		7
weight after lyophilization (mg)		145.4
residual water (%)		7.74
structure		malleable sponge
rehydration		nd

Table 4

HPMC E5	mg/cm²	7.95
	%	1.67
Cidofovir	mg/cm²	4.77
	%	1
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		159.6
residual water (%)		7.68

structure	malleable sponge
rehydration	nd

Table 5

HPMC E5	mg/cm²	3.97
	%	0.83
Cidofovir	mg/cm²	4.77
	%	1
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		115.17
residual water (%)		nd
structure		malleable sponge
rehydration		nd

Table 6

HPMC E5	mg/cm²	2.45
	%	1
Cidofovir	mg/cm²	0.36
	%	0.15
PEG 400	mg/cm²	0.49
	%	0.2
NaOH 2M (mg/cm²)		1.473
diameter lyophilisate (cm)		7.2
weight before lyophilization (g)		10
weight after lyophilization (mg)		200
residual water (%)		7.073
structure		malleable sponge
rehydration		very rapid

Table 7

HPMC E5	mg/cm²	3.14
	%	1
Cidofovir	mg/cm²	0.94
	%	0.30
PEG 400	mg/cm²	0.63
	%	0.2
NaOH 2M (mg/cm²)		3.77
diameter lyophilisate (cm)		4.5
weight before lyophilization (g)		5
weight after lyophilization (mg)		100
residual water (%)		8.63
structure		malleable sponge
rehydration		very rapid

Table 8

HPMC E5	mg/cm²	3.97
	%	0.83
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	1.98
	%	0.42
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		128.67
residual water (%)		6.0835
structure		malleable sponge
rehydration		nd

Table 9

HPMC E5	mg/cm²	6.36
	%	1.33
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	1.98
	%	0.42
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		166.485
residual water (%)		nd
structure		malleable sponge
rehydration		nd

Table 10

HPMC E5	mg/cm²	7.95
	%	1.43
Cidofovir	mg/cm²	4.77
	%	0.86
PEG 400	mg/cm²	1.98
	%	0.36
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		7
weight after lyophilization (mg)		166.6
residual water (%)		7.084
structure		malleable sponge
rehydration		nd

Table 11

HPMC E5	mg/cm²	7.95
	%	1.67
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	1.98
	%	0.42
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		183.695
residual water (%)		7.51
structure		malleable sponge
rehydration		nd

Table 12

HPMC E5	mg/cm²	7.95
	%	1.67
Cidofovir	mg/cm²	2.38
	%	0.5
PEG 400	mg/cm²	1.98
	%	0.42
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		151.24
residual water (%)		5.867
structure		malleable sponge
rehydration		nd

Table 13

HPMC E5	mg/cm²	11.93
	%	2.5
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	1.98
	%	0.42
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		236.74
residual water (%)		nd
structure		malleable sponge
rehydration		nd

Table 14

HPMC E5	mg/cm²	12.57
	%	1.67
Cidofovir	mg/cm²	1.88
	%	0.25
PEG 400	mg/cm²	2.51
	%	0.33
NaOH 2M (mg/cm²)		3.77
diameter lyophilisate (cm)		4.5
weight before lyophilization (g)		12
weight after lyophilization (mg)		nd
residual water (%)		nd
structure		malleable sponge
rehydration		nd

Table 15

HPMC E5	mg/cm²	15.91
	%	3.33
Cidofovir	mg/cm²	2.387
	%	0.5
PEG 400	mg/cm²	1.98
	%	0.42
NaOH 2M (mg/cm²)		6.127
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		246.91
residual water (%)		4.6845
structure		malleable sponge
rehydration		nd

Table 16

HPMC E5	mg/cm²	3.97
	%	0.83
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	3.97
	%	0.83
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		163.39
residual water (%)		6.92
structure		malleable sponge
rehydration		nd

Table 17

HPMC E5	mg/cm²	6.366
	%	1.33
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	3.97
	%	0.83
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		186.82
residual water (%)		4.935
structure		malleable sponge
rehydration		nd

Table 18

HPMC E5	mg/cm²	7.95
	%	1.67
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	3.97
	%	0.83
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		208.64
residual water (%)		5.2428
structure		malleable sponge
rehydration		nd

Table 19

HPMC E5	mg/cm²	7.95
	%	1.67
Cidofovir	mg/cm²	2.38
	%	0.50
PEG 400	mg/cm²	3.97
	%	0.83
NaOH 2M (mg/cm²)		6.167
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		176.275
residual water (%)		nd
structure		malleable sponge
rehydration		nd

Table 20

HPMC E5	mg/cm²	15.91
	%	3.33
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	3.97
	%	0.83
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		304.663
residual water (%)		5.6819
structure		malleable sponge
rehydration		nd

Table 21

HPMC E5	mg/cm²	7.95
	%	1.67
Cidofovir	mg/cm²	4.77
	%	1
PG	mg/cm²	1.98
	%	0.42
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		168.675
residual water (%)		nd
structure		slightly malleable sponge
rehydration		nd

Table 22

HPMC E5	mg/cm²	7.95
	%	1.67
Cidofovir	mg/cm²	2.38
	%	0.5
PG	mg/cm²	1.98
	%	0.42
NaOH 2M (mg/cm²)		6.167
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		139.05
residual water (%)		nd
structure		slightly malleable and brittle sponge
rehydration		nd

Table 23

HPMC E5	mg/cm²	7.95
	%	1.67
Cidofovir	mg/cm²	4.77
	%	1
PG	mg/cm²	3.97
	%	0.83
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		184.79
residual water (%)		nd
structure		slightly malleable and brittle sponge
rehydration		nd

Table 24

HPMC E15	mg/cm²	7.95
	%	1.67
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	1.98
	%	0.42
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		197.145
residual water (%)		nd
structure		malleable sponge
rehydration		nd

Table 25

HPMC 4000	mg/cm²	7.95
	%	1.67
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	1.98
	%	0.42
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		196.65
residual water (%)		nd
structure		malleable sponge
rehydration		nd

Table 26

HPMC 4000	mg/cm²	9.54
	%	2
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	1.98
	%	0.42
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		193.39
residual water (%)		nd
structure		malleable sponge
rehydration		nd

Table 27

HPMC 4000	mg/cm²	9.54
	%	2
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	3.97
	%	0.83
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		220.75
residual water (%)		3.35
structure		malleable sponge
rehydration		nd

Table 28

HPMC 4000	mg/cm²	1.98
	%	0.42
Cidofovir	mg/cm²	2.38
	%	0.5
PG	mg/cm²	3.97
	%	0.83
NaOH 2M (mg/cm²)		6.167
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		68.39
residual water (%)		nd
structure		malleable sponge
rehydration		nd

Table 29

HPMC K15	mg/cm²	7.95
	%	1.67
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	1.98
	%	0.42
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		194.39
residual water (%)		nd
structure		malleable sponge
rehydration		nd

Table 30

HPMC K15	mg/cm²	1.98
	%	0.42
Cidofovir	mg/cm²	2.38
	%	0.5
PG	mg/cm²	3.97
	%	0.83
NaOH 2M (mg/cm²)		6.167
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		68.05
residual water (%)		nd
structure		malleable sponge
rehydration		nd

Table 31

HEC 250M	mg/cm²	7.95
	%	1.67
Cidofovir	mg/cm²	2.38
	%	0.5
PEG 400	mg/cm²	1.98
	%	0.42
NaOH 2M (mg/cm²)		6.167
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		144.34
residual water (%)		nd
structure		malleable sponge
rehydration		very rapid

Table 32

HEC 250M	mg/cm²	9.54
	%	2
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	1.98
	%	0.42
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		200.56
residual water (%)		4.208
structure		malleable sponge
rehydration		very rapid

Table 33

HEC 250M	mg/cm²	9.54
	%	2
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	3.58
	%	0.75
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		221.69
residual water (%)		nd
structure		malleable sponge
rehydration		nd

Table 34

HEC 250 HX	mg/cm²	7.95
	%	1.67
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	1.98
	%	0.42
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		183.155
residual water (%)		3.44

structure	malleable sponge
rehydration	réhydratation immédiate

Table 35

HEC 250 HX	mg/cm²	7.95
	%	1.67
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	-
	%	-
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		149,95
residual water (%)		nd
structure		brittle sponge
rehydration		nd

Table 36

NaCMC	mg/cm²	4.77
	%	0.86
Cidofovir	mg/cm²	4.77
	%	0.86
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		7
weight after lyophilization (mg)		113.4
residual water (%)		8.265
structure		rigid and brittle sponge
rehydration		nd

Table 37

NaCMC	mg/cm²	4.77
	%	1
Cidofovir	mg/cm²	4.77
	%	1
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		113.4
residual water (%)		13.659
structure		very rigid and brittle sponge
rehydration		nd

Table 38

NaCMC	mg/cm²	3.18
	%	0.67
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	1.98
	%	0.42
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		157.335
residual water (%)		8.635
structure		rigid and brittle sponge
rehydration		nd

Table 39

NaCMC	mg/cm²	4.77
	%	0.86
Cidofovir	mg/cm²	4.77
	%	0.86
PEG 400	mg/cm²	1.98
	%	0.36
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		7
weight after lyophilization (mg)		133.25
residual water (%)		10.631
structure		rigid and brittle sponge
rehydration		nd

Table 40

NaCMC	mg/cm²	4.77
	%	1
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	1.98
	%	0.42
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		146.65
residual water (%)		6.51
structure		rigid and brittle sponge
rehydration		nd

Table 41

NaCMC	mg/cm²	6.36
	%	1.33
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	1.98
	%	0.42
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		149.65
residual water (%)		11.964
structure		very rigid and brittle sponge
rehydration		nd

Table 42

NaCMC	mg/cm²	4.77
	%	1
Cidofovir	mg/cm²	4.77
	%	1
PEG 400	mg/cm²	3.97
	%	0.83
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		169.05
residual water (%)		6.02
structure		very rigid and brittle sponge
rehydration		nd

Table 43

NaCMC	mg/cm²	3.18
	%	0.67
Cidofovir	mg/cm²	2.38
	%	0.5
PG	mg/cm²	3.97
	%	0.83
NaOH 2M (mg/cm²)		6.167
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		90.42
residual water (%)		nd
structure		slightly rigid and brittle sponge
rehydration		nd

Table 44

NaCMC	mg/cm²	4.77
	%	1
Cidofovir	mg/cm²	4.77
	%	1
PG	mg/cm²	3.97
	%	0.83
NaOH 2M (mg/cm²)		12.33
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		145.77
residual water (%)		nd
structure		very rigid and brittle sponge
rehydration		nd

Table 45

NaCMC	mg/cm²	4.77
	%	1
Cidofovir	mg/cm²	2.38
	%	0.5
PG	mg/cm²	3.97
	%	0.83
NaOH 2M (mg/cm²)		6.167
diameter lyophilisate (cm)		4
weight before lyophilization (g)		6
weight after lyophilization (mg)		108.08
residual water (%)		nd
structure		very rigid and brittle sponge
rehydration		nd

After rehydration, a gel-like composition can be obtained. A preferred gel-like composition typically comprises about 2% cidofovir and 4% HEC250M. The composition typically comprises HCL and NaOH in a quantity to reach a pH value of 7.2. The remaining component of the gel-like composition is water, to reach 100%. If required, a antibacterial agent could be added to further improve the shelf-life. One example is Benzyl alcohol (about 2%).

Example 5: water content in the sponge

The residual water in the sponge after lyophilization was measured by the Karl Fisher method (Metler DL35). From Tables 46 it can be seen that the percentage residual water varies in function of the polymer as well as in function of the presence of cidofovir.

Table 46

HPMC E5	PEG 400	PG	Cidofovir	% water	n
7.95	1.98			3,08 ± 1,36	3
6.36	3.97			1.95	1
7.95	3.97			1.83	1
15.91	3.97			3,18 ± 1,94	5
31.83	3.97			1,89 ± 0,401	3
7.95			4.77	7.68 ± 1,34	3
3.97	1.98		4.77	6.09 ± 0,35	2
7.95	1.98		2.38	5.87	1
7.95	1.98		4.77	7.39 ± 1,44	7
15.91	1.98		2.38	4.68	1
15.91	1.98		4.77	5.80 ± 1,08	2
3.97	3.97		4.77	6,91 ± 1,71	3
6.36	3.97		4.77	4.94 ± 0,5	2
7.95	3.97		4.77	5.25 ± 1,12	3
15.91	3.97		4.77	5,18 ± 0,67	4
7.95		1.98		2.69	1
10.58		2.63		3.33	1
11,93		2.94		3.18 ± 0,40	2
7.95		3.97		2.76	1
15.91		3.97		5.12	1
HEC 250 HX	**PEG 400**	**PG**	**Cidofovir**	**% water**	**n**
7.95	1.98			4,80 ± 1,4	15
7.95	1.98		4.77	3,44 ± 0,33	3
HEC 250 M	**PEG 400**	**PG**	**Cidofovir**	**% water**	**n**
9.54	1.98			4,44 ± 0,69	5
9.54	1.98		4.77	4,21 ± 1,26	4
HPMC 4000	**PEG 400**	**PG**	**Cidofovir**	**% water**	**n**

9.54	1.98			1,03 ± 0,06	3
9.54	3.97		4.77	3,35 ± 0,01	2
NaCMC	**PEG400**	**PG**	**Cidofovir**	**% water**	**n**
4,77	1,98			9,34 ± 1,98	3
5.57	1,98			8.19	1
4.77	3,97			6.86	1
4,77			4,77	11.86 ± 3,18	1
3.18	1.98		4.77	8.64 ± 0,02	2
4.77	1,98		4,77	6.51	1
6,36	1,98		4,77	11.96	1
4.77	3,97		4,77	6.02	1
4.77		1,98		8.33	1

The residual water content is lower in HPMC- and HEC-based sponges than in NaCMC-based sponges.

It has been established that the quantity of water which was added to obtain the aqueous composition before lyophilization (4, 5 or 6 ml were tested) did not influence the amount of residual water after lyophilization.

Example 6: uniformity of cidofovir

The uniformity of cidofovir presence inside the sponges has been tested by cutting sponges in 4 parts and testing the cidofovir dose by chromatography (HPLC). The conditions are as follows:

- stationary phase: LiChrospher® 100 RP-18 e (endcapped) (5 µm) in an analytical LiChrocart® column of 250 mm x 4 mm, d.i.
- mobile phase: HPLC buffer pH 6.5 / ACN (90/10, m/m)
- flow rate: 1 ml/min
- temperature: 30°C
- detection: spectrophotometric adsorption, UV 275 nm
- injection volume: 20 µl

The composition of the HPLC buffer is:

- 5 mM tetrabutyl ammonium hydrogen sulphate
- 5 mM ammonium dihydrogen phosphate
- pH adjusted to 6.5 with ammonium

Table 47 list the results for HPMC E5-based sponges, as a percentage of the theoretically expected dose.

Table 47

lot Nº	location	% of theoretical	lot Nº	location	% of theoretical
10C24-3	side	95.67	10C24-4	side	101.03
10C24-3	side	112.77	10C24-4	side	100.57
10C24-3	side	87.86	10C24-4	side	95.24
10C24-3	center	92.39	10C24-4	center	95.83

Example 7: stability of cidofovir

Lyophilized sponges have been bagged (PET/Al/EZ) and shielded from humidity, light and oxygen. The sponges were kept at three different temperatures: 4°C, 25°C and 45°C. Figure 1 shows the chromatograms of sponges that contained HPMC E5 (7.95 mg/cm²), PEG 400 (1.98 mg/cm²) and cidofovir (4.77 mg/cm²). Figures 1A, 1B, 1C, 1D, 1E, 1F and 1G represent respectively the chromatograms of the calibration (100 µg/ml), the sponge at T0 and the sponge at T1, T3, T6, T9 and T12 (i.e. respectively after one, three, six, nine and twelve month(s)) at 45°C. Figures 2A, 2B and 2C represent the chromatograms of a sponge comprising HEC 250HX (7.95 mg/cm²), PEG 400 (1.98 mg/cm²) and cidofovir (4.77 mg/cm²) respectively at T0, T1 and T3 (i.e. after one and three month(s)) at 45°C. It can be seen that the peak area is small (irrespective of the temperature at which the sponges are kept) and that the peak is equally present in the calibration chromatogram. Hence, the sponges are stable under the conditions tested.

Example 8: diffusion of cidofovir

Diffusion of cidofovir was measured with a Franz diffusion cell. A lyophilisate of 1.2 cm diameter was placed in the cell and rehydrated with 350 µl of a phosphate buffer (pH 5, 37°C). The diffusion kinetics are evaluated by HPLC in doses of 1 ml. Polymers were varied (HPMC E5, NaCMC, HEC 250HX and HEC 250M) as well as the presence or absence of plasticizer (PEG 400). The concentration of cidofovir was kept constant. From figures 3A, 3B and 3C, it seems that a plateau is reached for HPMC-based sponges after 1 hour, for NaCMC- and HEC 250M-based sponges after 2 hours and for

HEC 250HX-based sponges after 3 hours. Nevertheless, cidofovir diffuses fast from irrespective compositions.

Example 9: microscopic analysis

The structure of the sponges based on HPMC E5, HPMC 4000, HPMC K15 and HEC 250HX was evaluated by scanning electron microscopy. Pore structure was compared between sponges with different type of polymer, sponges with different concentration of the polymer, sponges with or without cidofovir, sponges with or without plasticizer as well as sponges with different plasticizer (PEG 400 or PG) and different concentrations of plasticizer.

Figures 4 to 8 are pictures of HPMC E5-based sponges at different magnitudes. From Figure 4, it is apparent that the external layer of PG-based sponges have more grooves than PEG 400-based sponges. From Figure 5, it is apparent that the pores are better organized in PEG 400-based sponges than in PG-based sponges. In confirmation that PEG 400-based sponges are more resistant, PG appears to lead to a disorganization of the pore structure, which pores become more fragile. The addition of cidofovir to the sponges likewise appears to make the pore structure more fragile. From Figure 6, it appears that the pore surface is smooth in the placebo sponges, whereas the surface is granular in sponges containing cidofovir, irrespective of whether PEG 400 or PG is used as plasticizer. It appears that cidofovir is distributed uniformly. From Figure 7, it is apparent that an increase in polymer concentration (from 7,95 to 15.91 mg/cm^2 HPMC E5) results in a more dense sponge structure with smaller pores. From Figure 8, it appears that the pore structure is better organized in the presence of PEG 400 plasticizer. In addition, it appears that less grooves are observed on the external layer of the sponge and that the pores are smaller by augmenting the concentration of the PEG 400 plasticizer. Figure 9 shows scanning electron microscopy images of sponges based on HPMC E5, HPMC 4000 or HPMC K15 without or with cidofovir. The molecular weight of the HPMC polymer appears to affect the pore size: the pores increase with the molecular weight of the polymer. Smaller pores are observed in the presence of cidofovir. The presence of cidofovir also results in less organized pores, which is even more pronounced in the sponges based on a higher molecular weight HPMC polymer.

Figures 10 and 11 represent pictures of sponges based on HEC 250HX polymer. From figure 10, it seems that the pores are smaller in the presence of cidofovir. From figure 11, it appears that the pore surface is smooth in the placebo sponges, whereas the surface is granular in sponges containing cidofovir. It appears that cidofovir is distributed as crystal form uniformly.

Example 10: porosity and sponge thickness

Experiments have been performed to evaluate the parameters which influence the thickness of the sponge. It has been found that increasing the quantity of the polymer in the aqueous composition before lyophilization only slightly increased the thickness of the sponge after lyophilization. On the other hand, the thickness of the sponge after lyophilization could be increased substantially by increasing the amount of water which is added to the mixture before lyophilization. Thicker sponges were obtained with a mixture of at least 8 g (8, 9, 12, or 15 g). Moreover, it appeared that PG-based compositions allowed for obtaining thicker sponges, whereas PEG 400-based compositions under the same conditions liquefied. A longer lyophilization cycle may be needed.

Example 11: viscosity

The viscosity of the sponges after rehydration was performed with a rheometer (ARES G2, TA instrument). The following parameters were kept constant for ease of comparison: sponge surface and amount of water for rehydration (900µl except for Figures 14 and 15). Measurements were performed at 37°C for 240 s at a rotation speed from 1 to 100 s^{-1}.

From Figure 12, it is clear that the NaCMC-based sponges are more viscous than the HPMC E5-based sponges. No difference in viscosity was observed between different HPMC E5 concentrations. HPMC E15-based sponges appeared more viscous than HPMC E5-based sponges (approaching the viscosity of NaCMC-based sponges). From Figure 13 it is clear that the viscosity is not influenced by the nature of plasticizer (PEG 400 or PG).

From Figures 14 and 15, it appears that the viscosity of the lyophilized compositions is not influenced by the lyophilization process. The viscosity of rehydrated HEC 250 M and HEC 250 HX based sponges is comparable to the viscosity of HEC 250 M and

HEC 250 HX gels (that were not lyophilized) and approaches the viscosity of a carbomer gel comprising cidofovir.

From Figure 16, it is clear that the viscosity is influenced by the type of polymer: HMPC K15, HPMC 4000 (with or without HPC GF), HEC 250 HX, HEC 250 M, HPMC E15 and HPMC E5 are classified following decreasing viscosity. Furthermore, the viscosity increases with increasing concentration of the polymer.

Example 12: sponge measurements by differential scanning calorimetry

The thermal transitions of lyophilisates and the eventual interaction between the polymers and cidofovir were evaluated by differential scanning calorimetry (DSC 25 Mettler Toledo, controlled by the TC15 TA Controller). The temperature was increased by 10°C per minute between 35 and 300°C. From Figure 17, it is clear that cidofovir dehydrated in powder form shows an endothermal peak at 280°C. This peak is absent in the calorimetrical analysis of the different sponges because cidofovir is transformed to salt by the method for producing the lyophilized composition.

Claims

1. A sheet-shaped lyophilized composition comprising
(a) cidofovir in an amount between 0.1 and 5 mg/cm^2;
(b) hydroxyethylcellulose (HEC) or hydroxypropylmethylcellulose (HPMC) in an amount between 1 and 17 mg/cm^2; and optionally
(c) a plasticizer in an amount between 0 and 5 mg/cm^2.

2. The lyophilized composition according to claim 1 comprising:
(a) cidofovir in an amount between 0.1 and 5 mg/cm^2;
(b) hydroxyethylcellulose (HEC) in an amount between 1 and 17 mg/cm^2; and
(c) a plasticizer in an amount between 0.5 and 4 mg/cm^2.

3. The lyophilized composition according to claim 1 or 2, wherein HEC is present in an amount between 7 and 10.5 mg/cm^2, between 5 and 10 mg/ cm^2, between 8 and 10 mg/ cm^2, or between 10 and 16 mg/ cm^2.

4. The lyophilized composition according to anyone of claims 1 to 3, wherein said HEC is selected from the group consisting of HEC H4000, and HEC 250HHX, preferably HEC 250M, HEC 250HX, preferably HEC 250 M or HX.

5. The lyophilized composition according to any one of claims 1 to 4, which presents itself as a high viscosity composition.

6. The lyophilized composition according to claim 1 comprising:
(a) cidofovir in an amount between 0.1 and 5 mg/cm^2;
(b) hydroxypropylmethylcellulose (HPMC) in an amount between 1 and 17 mg/cm^2; and
(c) a plasticizer in an amount between 0 and 5 mg/cm^2.

7. The lyophilized composition according to claim 6, wherein HPMC is present in an amount between 2.5 and 8 mg/ cm^2, between 4 and 8 mg/ cm^2 or between 8 and 15 mg/ cm^2.

8. The lyophilized composition according to claim 6 or 7 wherein said HPMC is selected from the group consisting of HPMC E5, HPMC E15, HPMC 4000 and HPMC K15, preferably HPMC E5 or HPMC E15

9. The lyophilized composition according to any one of claims 6 to 8, which presents itself as a low viscosity composition.

10. The lyophilized composition according to claim 1 to 7, wherein said plasticizer is selected from the group consisting of polyethylene glycol 400 (PEG 400), polyethylene glycol 4000 (PEG 4000) and propylene glycol (PG), preferably PEG 400.

11. The lyophilized composition according to any of claims 1 to 8, wherein water is present in an amount between 1 and 10 weight%, and/or NaOH is present in an amount of between 0.15 and 0.25 weight%.

12. The lyophilized composition according to any of claims 1 to 11, wherein said composition is a porous malleable matrix.

13. The lyophilized composition according to any of claims 1 to 12 for use in medicine, preferably for use in medicine, preferably for use in treating infection with human DNA-virus such as Herpes virus, Pox virus, Papilloma virus, Adenovirus, Smallpox virus, or Human papillomavirus (HPV), or accompanying pathologies such as those selected from the group comprising: virus induced lesions or warts of the vagina, cervix, anogenital region, mucosa, epithelium of the oral sphere, or skin, precancerous lesions and/or neoplasms or cancers caused by HPV infection, more specifically to the cervix or the mucosal surface of the cervix.

14. A gel-like composition obtained after rehydration of the lyophilized composition according to any of claims 1 to 12 for use in medicine, preferably for use in treating infections with human DNA-viruses such as Herpes, Pox, Papillomavirus, Adenoviruses, Smallpox viruses, or Human papillomavirus (HPV) infection or accompanying pathologies such as those selected from the group comprising: virus induced lesions or warts of the vagina, cervix,

anogenital region, mucosa, epithelium of the oral sphere, or skin, precancerous lesions and/or neoplasms or cancers caused by viral infection, more specifically to the cervix or the mucosal surface of the cervix.

15. The lyophilized composition according to any of claims 2 to 5 for use in treating human DNA virus infection or accompanying pathologies, wherein the composition is rehydrated before administration.

16. The lyophilized composition according to any one of claims 6 to 9, for use in treating human DNA virus infection or accompanying pathologies , wherein the composition is not rehydrated before administration.

17. The lyophilized composition according to any of claims 1 to 16, or the gel-like composition of claim 14, for use in topical drug delivery to the vagina, cervix, anogenital region, mucosa, epithelium of the oral sphere, or skin.

18. A method for producing the lyophilized composition according to any of claims 1 to 12, comprising the steps of
(a) dispersing HEC or HPMC in water to obtain a homogenized composition;
(b) optionally dispersing plasticizer in the composition obtained in step (a) to obtain a homogenized composition;
(c) dispersing cidofovir and optionally adding NaOH 2M solution in the composition obtained in step (b) or alternatively the composition obtained in step (a) if no plasticizer is added to obtain a homogenized composition;
(d) lyophilizing the composition obtained in step (c),
wherein plasticizer needs to be added in step (b) when HEC is used in step (a).

19. The method according to claim 18, wherein the homogenized composition of step (c) comprises between 1 and 17 mg/cm^2 of HEC or HPMC.

20. The method according to any of claims 17 to 18, wherein the homogenized composition of step (c) comprises between 7 and 10.5 mg/cm^2, between 5 and 10 mg/cm^2, between 8 and 10 mg/cm^2, or between 10 and 16 mg/cm^2 HEC and between 1.5 and 33 mg/cm^2 plasticizer.

21. The method according to any of claims 17 to 19, wherein the homogenized composition of step (c) comprises between 2.5 and 8 mg/cm², between 4 and 8 mg/cm², or between 8 and 15 mg/cm² HPMC and between 0 and 5 mg/cm² plasticizer.

22. The method according to any of claims 17 to 21, wherein the homogenized composition of step (c) comprises between 0.1 and 5 mg/cm² cidofovir.

23. The method according to any of claims 17 to 22, wherein the homogenized composition of step (c) has a pH between 6 and 8, preferably between 6.5 and 7.5.

24. The method according to claim 23, wherein the homogenized composition of step (c) comprises NaOH.

25. A method of preparing a gel-like composition according to claim 14, comprising the step of rehydrating the lyophilized composition according to any of claims 1 to 12.

26. A sheet-shaped solid porous malleable matrix obtained by lyophilization of an aqueous composition, said aqueous composition comprising between 1 and 17 mg/cm² HEC or HPMC, between 0 and 5 mg/cm² plasticizer, and between 0.1 and 5 mg/cm² cidofovir, wherein between 1.5 and 3 mg/cm² of said plasticizer is present when HEC is used.

27. The matrix according to claim 25, wherein said aqueous composition comprises:
- between 7 and 10.5 mg/cm², between 5 and 10 mg/ cm², between 8 and 10 mg/ cm², or between 10 and 16 mg/ cm² HEC,
- between 1.5 and 3mg/cm² plasticizer, and
- between 0.1 and 5 mg/cm² cidofovir.

28. The matrix according to claim 25, wherein said aqueous composition comprises:
- between 2.5 and 8 mg/ cm², between 4 and 8 mg/ cm² or between 8 and 15 mg/ cm² HPMC,
- between 0 and 5 mg/cm² plasticizer, and

- between 0.1 and 5 mg/cm^2 cidofovir.

29. A drug delivery applicator, comprising the sheet-shaped lyophilized composition according to any of claims 1 to 12 or the matrix according to any of claim 25 to 27.

30. The drug delivery applicator according to claim 28, in the form of a cervical cap or a cervix-covering pessary, (5 to 15) optionally comprising a drug-impermeable barrier preventing the active ingredient form diffusing, or in the form of a vaginal inserter, a vaginal cream inserter, or a tampon inserter.