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Research Paper

# Chitosan–tripolyphosphate nanoparticles as a possible skin drug delivery system for aciclovir with enhanced stability

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## Abstract

**Objectives** The aim of the present study was to create a skin delivery system based on chitosan–tripolyphosphate nanoparticles for aciclovir with enhanced chemical stability.
**Methods** Nanoparticles were formed spontaneously using ionotropic gelation with tripolyphosphate. Two different sizes of aciclovir-loaded nanoparticles were characterised in terms of zeta potential, particle size and polydispersity index.
**Key findings** Standard diffusion experiments using Franz-type diffusion cells showed reasonable skin permeability that depended on particle size and chitosan content. The larger the nanoparticle, having a higher chitosan content, the better the aciclovir permeation through porcine skin. Differential scanning calorimetry studies showed a remarkable decrease in the typical transition temperature, indicating an interaction between skin lipid bilayer and the nanoparticles. Moreover, the chemical stability of aciclovir was significantly increased by the nanoparticle system. After the observation period of 5 weeks, aciclovir incorporated into nanoparticles had undergone photo-oxidation to a significantly lower extent than pure aqueous solution. This degradation product of aciclovir was analysed using LC/MS, and its identity established.
**Conclusions** These studies demonstrate that incorporation of aciclovir into chitosan–tripolyphosphate nanoparticles significantly improves its chemical stability. Moreover, skin diffusion studies *in vitro* showed improved permeation of aciclovir from the nanoparticle system, especially from nanoparticles with higher chitosan content.
**Keywords** chitosan-tripolyphosphate nanoparticles; skin diffusion; aciclovir; differential scanning calorimetry; LC/MS

## Introduction

Chitosan is a natural cationic polymer obtained by deacetylation of chitin using chemical or enzymatic reactions. In the usual sense, chitosan is chitin with more than 40% deacetylation. From the chemical point of view, it is a polycationic copolymer consisting of $\beta$-(1–4)-linked glucosamine and *N*-acetylglucosamine units. Chitosan is increasingly favoured in various fields of drug delivery for its biological properties such as biocompatibility and non-toxicity. Chitosan is metabolised by lysozyme, which is able to break the chitosan into smaller units.[1,2] Chitosan also possesses mucoadhesive, wound-healing and antimicrobial properties.[3,4]

The presence of reactive amino groups means that chitosan can be modified easily to create micro- and nanoparticles or porous hydrogels.[5] Chitosan microparticles and nanoparticles have been made by chemical cross-linking with different kinds of polyanions such as glycyrrhetic acid, polyaspartic acid and other oppositely charged molecules, yielding controlled-drug-release formulations with less toxicity.[6,7] One of the most extensively used polyanions is tripolyphosphate (TPP). The interaction of chitosan with TPP leads to the formation of biocompatible nanoparticles. The cross-linking density, crystallinity and hydrophilicity of cross-linked chitosan allow modulation of drug release and extends its range of potential applications in drug delivery.[8]

Given the successful use of chitosan-TPP (CS–TPP) nanoparticles through different barriers in recent years, we investigated whether they may also be useful for skin drug delivery.[9,10] The aims of the present study were therefore to create a CS–TPP drug delivery

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system for aciclovir (model drug) to improve its permeability through the skin, to enhance the chemical stability of aciclovir by incorporation into CS–TPP nanoparticles, and to characterise the physicochemical properties of the new delivery system.

## Materials and Methods

### Materials

Chitosan (<500 kDa) in powdered form was a gift from Syntapharm (Mülheim, Germany). The degree of deacetylation was determined by NMR as 95%.[11] TPP and methanol were from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Aciclovir was purchased from Fagron GmbH (Barsbüttel, Germany).

### Preparation of chitosan–tripolyphosphate nanoparticles

CS–TPP nanoparticles were prepared using ionotropic gelation between positively charged amino groups of chitosan and negatively charged TPP, as reported previously by Krauland and Alonso M.[12] Briefly, 0.1 g or 0.2 g chitosan was dissolved in 100 ml distilled water in the presence of 1 M HCl (which is essential to achieve protonation of amino groups), under magnetic stirring overnight at room temperature.[13] TPP aqueous solution (1 ml of about 0.7 mg/ml) was added to various volumes of 0.1% and 0.2% chitosan solution under gentle stirring at room temperature in order to obtain different chitosan : TPP (v/v) ratios. The final ratios were obtained by mixing 1 ml TPP with 3.5 ml 0.1% chitosan or 3.5 ml 0.2% chitosan solution for 10 min. The nanoparticles were formed spontaneously and were then concentrated by centrifugation at 24 562*g* for 30 min at 25°C in a glycerol bed (10 $\mu$l glycerine). The supernatants were discarded and the CS–TPP nanoparticles were resuspended in 100 $\mu$l purified water.[12] The final pH value of all tested solutions was between 5.3 and 5.5.

For incorporation of aciclovir into the CS–TPP nanoparticles, aciclovir was directly dissolved in the acidic chitosan solution at a concentration of 0.45 mg/ml, following by the procedure described above.

### Effect of pH on formation of nanoparticles

The pH of the chitosan solution was adjusted to values in the range 3.5 to 6.5 by addition of 0.1 mM HCl or NaOH. Different batches of CS–TPP nanoparticles were produced by combining these chitosan solutions with aqueous TPP solutions. Each experiment was repeated five times.

### Physicochemical characterisation of nanoparticles

The particle size and polydispersity index (PDI) of the CS–TPP nanoparticles were determined by photon correlation spectroscopy; the zeta potential was determined by electrophoretic mobility using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). For particle size analysis and the determination of electrophoretic mobility, samples were diluted with filtered distilled water ($n$ = 5).

For calculation of the yield, a fixed volume of nanoparticles was centrifuged at 24 562*g* for 40 min at room temperature without a glycerol bed. The supernatants were discarded and the microtubes containing the centrifugates were frozen at −20°C. The samples were then freeze-dried at −30°C for 3 h followed by −55°C overnight. After thawing the samples for 2 h at room temperature, the yield was determined by calculating the ratio of the actual weight and theoretical weight of the nanoparticles ($n$ = 3).[14]

### Determination of loading capacity and encapsulation efficiency

To determine loading capacity and encapsulation efficiency, aciclovir-loaded nanoparticles were isolated by centrifugation as described above. The amount of unbound aciclovir in the discarded supernatant was measured by HPLC using a diode array detector (series 200 LC; Perkin Elmer, Norwalk, CT, USA) at a wavelength of 252 nm. Briefly, supernatants ($n$ = 3) were diluted with 1 N $CaCl_2$ and then centrifuged at 24 562*g* for 10 min in order to precipitate polymer residue (we confirmed that the treatment with 1 N $CaCl_2$ did not decrease the aciclovir concentration significantly). After filtration (0.45 $\mu$m pore size), 20 $\mu$l of the supernatant was injected onto the system using an autosampler (series 200 Perkin Elmer). Separation was achieved using a C-18 RP column (Nucleosil 100-5, 250 mm × 4 mm, Macherey-Nagel, Düren, Germany) at 40°C. The mobile phase consisted of methanol/water 10/90.[15] The flow rate was maintained at 1.0 ml/min and the injection volume was 20 $\mu$l. The aciclovir concentration used to construct the calibration curve was in the range from 0.05 to 10 $\mu$g/ml ($n$ = 7). Loading capacity was calculated as [(total amount of drug − amount of unbound drug)/weight of nanoparticles] × 100. Encapsulation efficiency of aciclovir was calculated from [(total amount of drug − amount of unbound drug)/total amount of drug] × 100.

### Physicochemical stability of nanoparticles at room temperature

The different batches of CS–TPP nanoparticles with and without aciclovir were stored at room temperature for an observation period of 6 weeks. Particle size and zeta potential were monitored weekly using a Zetasizer (Nano ZS; Malvern Instruments) ($n$ = 5).

### Chemical stability of aciclovir in nanoparticles

The chemical stability of aciclovir incorporated into CS–TPP nanoparticles was compared with its stability in aqueous solution. The concentrations of aciclovir in CS–TPP nanoparticles and control sample were the same. The samples were stored in Eppendorf tubes under daylight exposure at air-conditioned room temperature (approximately 22°C). HPLC analysis was performed weekly for 5 weeks ($n$ = 3).

To characterise the main degradation product of aciclovir, an aqueous solution of aciclovir that had been stored in an Eppendorf tube under daylight exposure at room temperature for 2 weeks was analysed by LC/MS. The analysis was performed on an UltiMate 3000 RSLC series system (Dionex, Germering, Germany) coupled to an electrospray ionisation–3D-ion-trap mass spectrometer (ESI-IT-MS) (HCT, Bruker Daltonics, Bremen, Germany). Ten microlitres of the sample was loaded onto a C-18 RP column (Nucleosil 100-5, 250 mm × 4 mm; Macherey-Nagel) and eluted into the

orthogonal ESI source using a 10 min linear gradient of 0–10% methanol (HPLC grade) in 5 mM ammonium acetate pH 7.5 at a flow of 1.0 ml/min. The mass spectrometer was operated in automated data-dependent acquisition mode where each MS scan (*m/z* 50–400, average of five spectra) was followed by an MS/MS scan (*m/z* 15–300, average of five spectra, isolation window 4 Th) of the most intense precursor ion. Helium was used as the collision gas, and the fragmentation amplitude was 0.40 V in positive-ion mode and 0.60 V in negative-ion mode. Source parameters were as follows: capillary voltage: 4.0 kV, nebuliser (nitrogen): 60 psi; dry gas flow (nitrogen): 12 l/min; dry temperature: 365°C. For comparison, freshly prepared aqueous solutions of aciclovir and guanine hydrochloride were also analysed. The aciclovir solution containing the degradation product was re-analysed after incubation with 1 M HCl overnight at 37°C.

### In-vitro permeation studies

In-vitro permeation studies with porcine abdominal skin were performed as reported previously.[16] About 1 ml of aciclovir-loaded 0.1% or 0.2% CS–TPP nanoparticles was applied to the skin surface. Prior to quantification, the permeated samples were diluted with 1 N $CaCl_2$, centrifuged at 17 586*g* for 6 min and filtered (pore size 0.45 $\mu$m). Each batch was quantified in quadruplicate using the HPLC method described above. As a control we used aciclovir dissolved in water at the same concentration as in the nanoparticles (0.45 mg/ml). The pH value of all applied solutions was approximately 5.4.

### Differential scanning calorimetry

Differential scanning calorimetry (DSC) profiles of thawed and additionally dabbed porcine epidermis were obtained using a Perkin-Elmer DSC 7 differential scanning calorimeter. Sliced skin samples (1 $cm^2$) were impregnated for 14 h with 2 ml water (control) or 0.1% or 0.2% CS–TPP nanoparticle formulations. The sample weight was in the range 17–20 mg. The samples were placed in aluminium pans and sealed hermetically, than heated from 30 to 120ºC at a constant rate of 5ºC/min under a continuous flow of dry nitrogen. Transition temperatures of the thermograms are taken from peak maxima values and from onset temperatures. Gravimetric analysis of untreated skin samples prior to DSC experiments showed average water content of about 55.7 ± 1.44% (w/w). Treated samples absorbed about 10 ± 7% (w/w) of used impregnation.

### Statistical analysis

Results are given as means ± SD of at least three experiments. Data were exported to the GraphPad Prism statistics software. Analysis groups consisted of independent mean values; the Gaussian distribution of the data was verified using the Kolmogorov–Smirnov test. Statistical analysis was performed by one-way analysis of variance followed by Dunnet's or Tukey's post-hoc tests. Values for loading capacity and encapsulation efficiency and results of diffusion studies were analysed using the paired Student's *t*-test. $P < 0.05$ was considered significant.

## Results

### Preparation and physicochemical characterisation of pure nanoparticles and pH effect

CS–TPP nanoparticles were established only with particular chitosan : TPP ratios (Table 1). Regarding the entanglement of chitosan chains with TPP to form particles, a higher content of chitosan resulted in formation of significantly larger nanoparticles.[17] This was confirmed by our results (Table 1).

Table 2 shows that the pH of chitosan solution influenced the particle size and PDI values. The size of the nanoparticles did not show any significant difference in the pH range from 3.5 to 4.9. From pH 4.9 to 6 they showed remarkable swelling behaviour ($P < 0.05$), and aggregated at pH values higher than 6, where the chitosan became completely uncharged. The

**Table 1** Physicochemical properties of chitosan–tripolyphosphate nanoparticles prepared with different ratios (v/v) of 0.1% and 0.2% chitosan and tripolyphosphate

| CS : TPP ratio (v/v) | Mean particle size (nm) | Polydispersity index | Zeta potential (mV) | Yield (%) |
|---|---|---|---|---|
| **0.1% chitosan** | | | | |
| 2/1 | Precipitation | – | | |
| 3/1 | Precipitation | – | | |
| 4/1 | Precipitation | – | | |
| **4.5/1** | **350 ± 20** | **0.14–0.24** | +31 ± 2 | 25.9 ± 1.1 |
| 5/1 | 368 ± 20* | 0.21–0.23 | | |
| 5.5/1 | 390 ± 10* | 0.22–0.27 | | |
| 6/1 | 510 ± 10* | 0.22–0.27 | | |
| **0.2% chitosan** | | | | |
| 2/1 | Precipitation | – | | |
| 3/1 | Precipitation | – | | |
| **4/1** | **450 ± 50** | **0.4–0.52** | | |
| 4.5/1 | 634 ± 38** | 0.12–0.24 | +31 ± 2 | 40.3 ± 0.8 |
| 5/1 | 600 ± 50** | 0.6–0.8 | | |
| 5.5/1 | 700 ± 10** | 0.9–1.0 | | |

Values are means ± SD from at least five experiments. CS, chitosan; TPP, tripolyphosphate. Significantly larger particles compared with the *4.5/1 or **4/1 nanoparticles (Dunnet's test).

**Table 2** Effect of pH on nanoparticle distribution of 0.1% chitosan–tripolyphosphate nanoparticles

| pH | Mean particle size (nm) | Polydispersity index | Zeta potential (mV) |
|---|---|---|---|
| 3.5 | 330 ± 20 | 0.27–0.29 | 29 ± 1.1 |
| 3.5 | 330 ± 20 | 0.27–0.29 | |
| 4 | 360 ± 21 | 0.25–0.26 | 21 ± 2.1 |
| 4.5 | 367 ± 14 | 0.28–0.30 | 21 ± 0.5 |
| **4.9** | **350 ± 21** | **0.14–0.24** | **31 ± 2.0** |
| 5.5 | 500 ± 23* | 0.40–0.44 | 22 ± 1.0 |
| 6 | 520 ± 26* | 0.35–0.36 | 22 ± 0.8 |
| 6.5 | Precipitation | – | – |

Values are means ± SD from five experiments. *$P < 0.05$ (Tukey's test).

interaction between chitosan and TPP was completely destroyed at pH values lower than 3.5, resulting in the dissolution of nanoparticles. The zeta potential of successfully produced nanoparticles was positive, in the range 22 to 30 mV.

## Encapsulation of aciclovir within nanoparticles and physicochemical properties of nanoparticles

Incorporation of aciclovir, a poorly soluble substance (1.3 mg/ml in water at 25°C), into the CS–TPP nanoparticles was optimised by dissolving aciclovir directly in the chitosan solution at a concentration of 0.45 mg/ml. However, an increase in particle size with higher chitosan content did not result in significantly higher loading capacity or encapsulation efficiency (Table 3).

## Physicochemical stability of nanoparticles at room temperature

CS–TPP nanoparticle formulations were unstable at room temperature in terms of mean particle size. Cuna *et al.* explained this property as colloidal system tendency to aggregate.[14]

Our experiments verified this fact for both 0.1% (Figure 1) and 0.2% chitosan (Figure 2) nanoparticles. Aggregation ability was much higher in 0.2% CS–TPP nanoparticles, showing a significant increase in particle size compared with 0.1% CS–TPP nanoparticles at each weekly measurement. The particle size of the latter was significantly increased after 5 weeks. The incorporation of aciclovir into 0.2% CS–TPP nanoparticles resulted in physical breakdown after 2 weeks (Figure 2). However, we successfully incorporated aciclovir into the formulation with 0.1% chitosan; the physical stability of these did not decrease within 6 weeks, evidenced by the stable zeta potential (Figure 1).

## Chemical stability of aciclovir in nanoparticles

Aciclovir is an important antiviral drug used in the treatment of many types of herpes infections. However, it has been found that aciclovir degrades under exposure to light, oxidants, temperature and in different solvents.[15]

The HPLC analysis clearly shows a degradation product at a retention time of about 1.5 min (Figure 3), which is in agreement with literature data, where the photolytic degradation of aciclovir to a non-identified chromophoric compound is explained.[15]

According to the results of the degradation studies under daylight exposure at room temperature, 12% of aciclovir in CS–TPP nanoparticles degraded after 5 weeks whereas 12% of aciclovir in water had decomposed within 2 weeks. To further characterise the photolytic degradation products of aciclovir,

**Table 3** Physicochemical characteristics of aciclovir-loaded 0.1% and 0.2% chitosan–tripolyphosphate nanoparticles

| | 0.1% chitosan | 0.2% chitosan |
|---|---|---|
| CS : TPP ratio (v/v) | 4.5/1 | 4.5/1 |
| Mean particle size (nm) | 380 ± 50 | 703 ± 15 |
| Polydispersity index | 0.11–0.21 | 0.23–0.28 |
| Zeta potential (mV) | +30 ± 3 | +27 ± 3 |
| Loading capacity (%) | 6.02 ± 0.4 | 6.23 ± 0.5 |
| Encapsulation efficiency (%) | 13.56 ± 0.6 | 14.06 ± 0.4 |
| Yield (%) | 32.6 ± 2.3 | 44.5 ± 2 |

The total amount of aciclovir is 0.45 ± 0.05 mg/ml of chitosan (CS)–tripolyphosphate (TPP) solution. Values are means ± SD of at least five experiments.

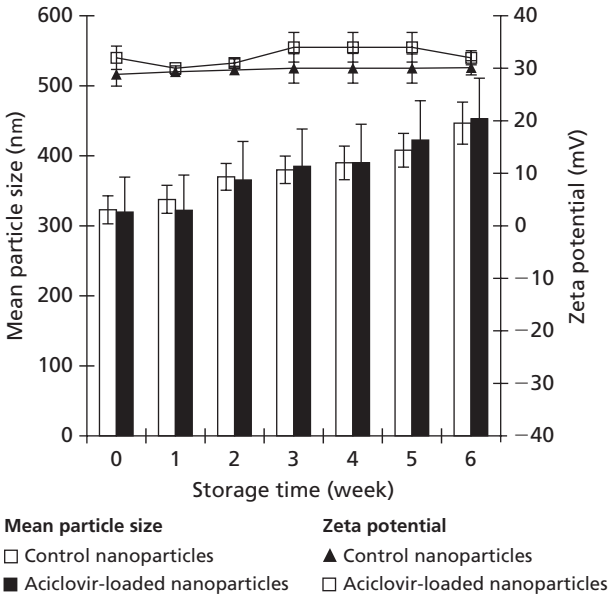


**Figure 1** Comparison of the physicochemical stability of 0.1% chitosan–tripolyphosphate nanoparticles with and without aciclovir. Values are means ± SD of at least five experiments.

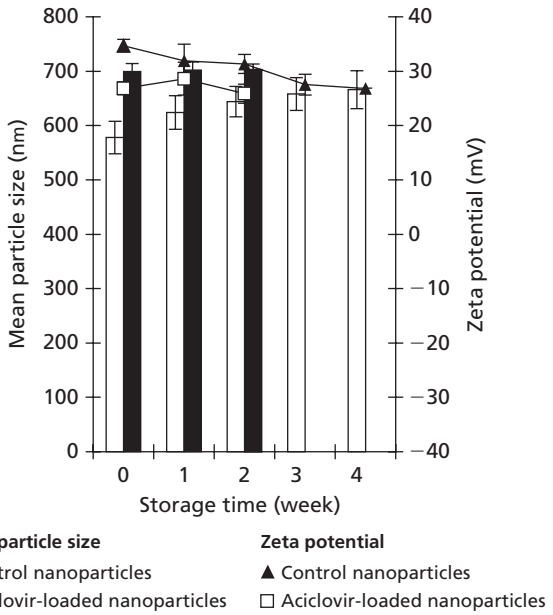


**Figure 2** Comparison of the physicochemical stability of 0.2% chitosan–tripolyphosphate nanoparticles with and without aciclovir. Values are means ± SD of at least five experiments.

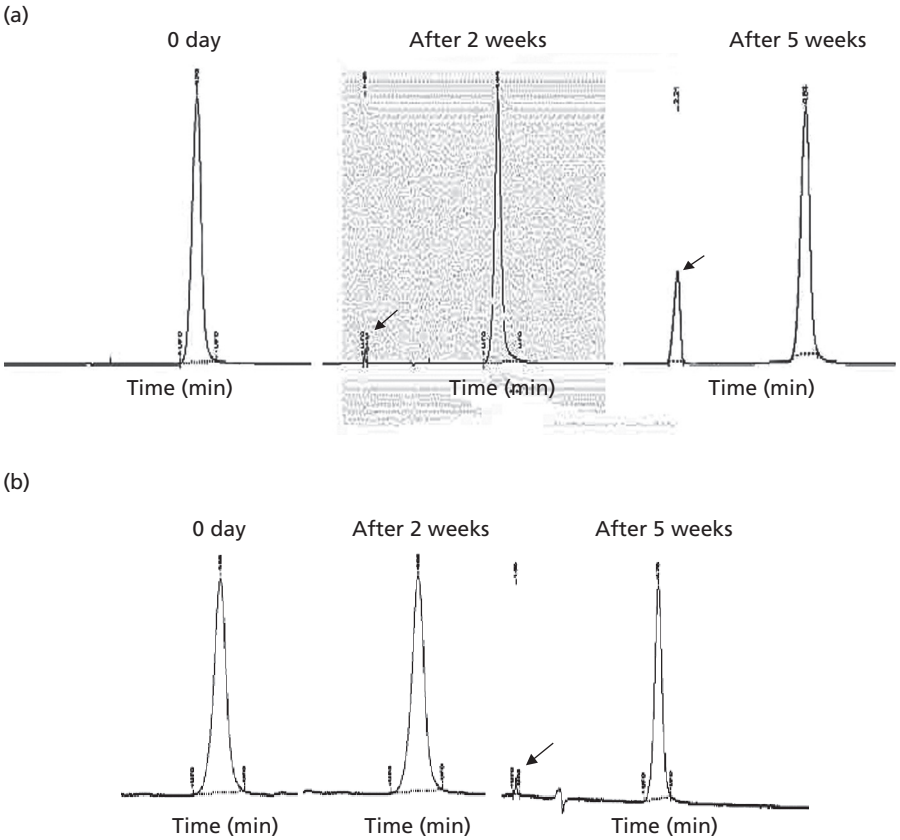


**Figure 3** Chromatograms showing the photolytic degradation of aciclovir in (a) water and (b) in chitosan–tripolyphosphate nanoparticles after 2 and 5 weeks

LC/MS analysis was performed. Whereas in freshly prepared aqueous solutions only aciclovir (3) is detected (Figure 4a), a prominent degradation product (1) is formed following 2 weeks' light exposure at room temperature (Figure 4b). This degradation product is different from guanine (2), which is formed by acid-catalysed hydrolytic cleavage of the $N_9$-linked (2-hydroxyethoxy) methyl chain upon incubation with 1 M HCl overnight at 37°C (Figure 4c). The molecular weight of the degradation product (239.1 Da) is 14 Da higher than the mass of aciclovir (225.1 Da). From the comparison of the $MS^2$ spectra of aciclovir (Figures 5a and 5c) and the degradation product (Figures 5b and 5d), it can be concluded that photo-oxidation of the 2-hydroxyethoxy group has occurred. Since the mass spectrometric response of both species in positive- and negative-ion mode is comparable, the photo-oxidation product is likely to be 9-((2-hydroxyacetyl) methyl) guanine.

## Skin diffusion

In the in-vitro permeation studies, two different sizes of CS–TPP nanoparticles containing aciclovir were applied to porcine skin for 48 h. The permeation of aciclovir was proportional to the nanoparticle size, regulated by the amount of chitosan. In other words, larger particle size improved the diffusion of aciclovir through the epidermis. The permeation of aciclovir from larger particles was about 1.5-fold higher than from smaller nanoparticles (Figure 6; $P < 0.05$).

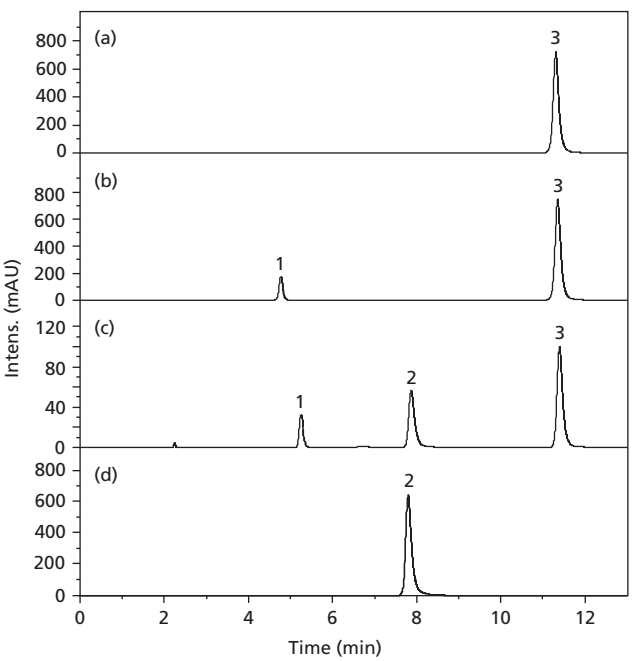


**Figure 4** HPLC chromatograms (UV trace at 252 nm) of aqueous solutions of (a) freshly prepared aciclovir, (b) aciclovir stored in an eppendorf tube under light exposure at room temperature for 2 weeks and (c) additionally incubated with 1 M HCl overnight at 37°C, and (d) guanine hydrochloride.

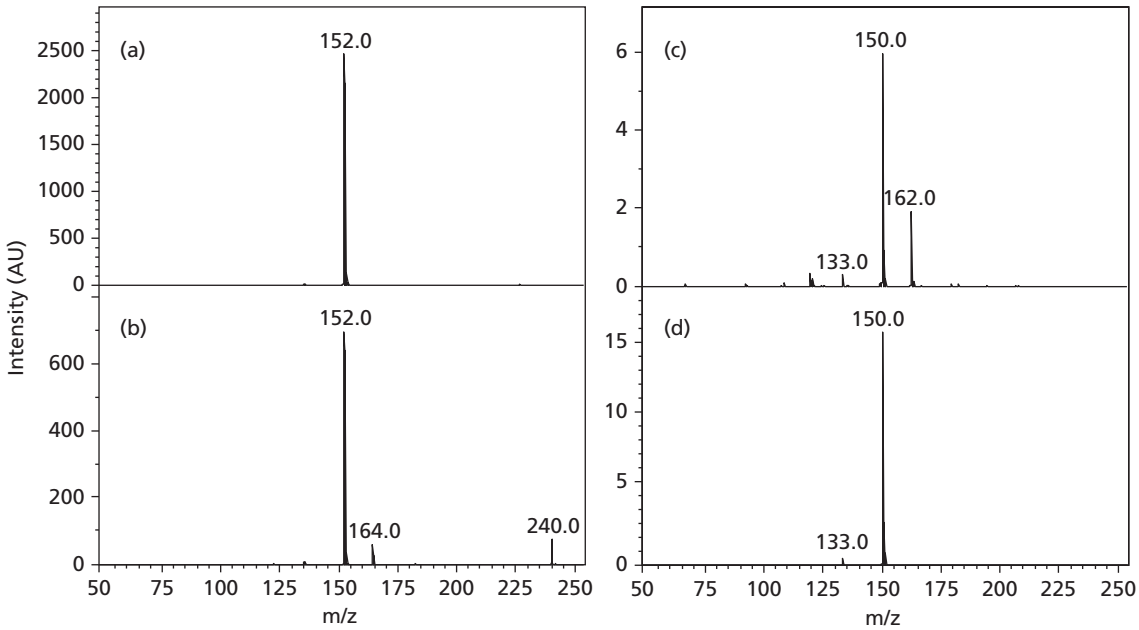


**Figure 5** Electrospray ionisation–3D-ion-trap mass spectrometry spectra of (a) the $[M+H]^+$ ion of aciclovir (226.0→), (b) the $[M+H]^+$ ion of the photo-oxidation product (240.0→), (c) the $[M-H]^-$ ion of aciclovir (224.1→), and (d) the $[M-H]^-$ ion of the photo-oxidation product (238.1→). The main product ions of aciclovir originate from the elimination of the $N_9$-linked (2-hydroxyethoxy)methyl-chain (*m/z* 152.0 or *m/z* 150.0) and the elimination of the 2-hydroxyethoxy group (*m/z* 162.0). In the case of the photo-oxidation product, the fragment ions are interpreted as the elimination of $N_9$-linked (2-hydroxyacetyl)methyl-chain (*m/z* 152.0 or *m/z* 150.0) and the elimination of the 2-hydroxyacetyl group (*m/z* 164.0).

The diffusion profile of aciclovir from CS–TPP nanoparticles through the epidermis was biphasic, reflecting the initial fast partitioning of aciclovir close to the nanoparticle surface, followed by slower diffusion of the drug trapped inside the nanoparticle matrix.[17] According to Figure 6, aciclovir permeation after 8 h was approximately 50% of the drug penetration over 48 h. Therefore, it might be possible to control the penetration of aciclovir by the use of CS–TPP nanoparticles.

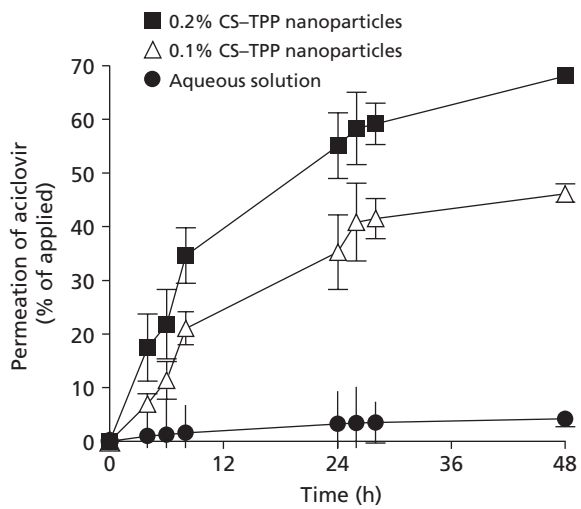


**Figure 6** Comparison of aciclovir permeation through porcine skin from different chitosan–tripolyphosphate nanoparticles and aciclovir aqueous solution. Graph shows means ± SD of four experiments. CS–TPP, chitosan–tripolyphosphate.

### Differential scanning calorimetry studies

DSC thermograms of porcine skin with and without treatment are shown in Figure 7. It is obvious that the different impregnation types resulted in different thermograms. Porcine skin has one endothermic peak at around 78°C, which corresponds to intracellular lipid layers.[18–20] Treatment of the skin with water or nanoparticle formulations resulted in a shift to significantly lower temperatures ($P < 0.05$). The maximum transition temperature (peak maxima value) was shifted from 78°C for untreated skin to 66.5°C after treatment with 0.2% CS–TPP nanoparticles (Figure 7). Moreover, a comparison of skin impregnated with 0.2% and 0.1% chitosan nanoparticles still indicates a significant shift in onset temperature ($P < 0.05$) (Table 4).

## Discussion

CS–TPP nanoparticles were formed by means of electrostatic interaction between the positively charged $-NH_2$ groups of chitosan in acidic medium (pH 4.9) and negatively charged phosphate and hydroxide groups of TPP aqueous solution, which compete with each other to interact with $-NH_3^+$ sites of chitosan.[8]

The nanoparticles were positively charged over the chitosan : TPP ratio used, reflecting the excess of chitosan amino groups, which may be favourable for drug delivery because of enhanced interaction with negatively charged groups on the skin surface. It was also evident that the pH of

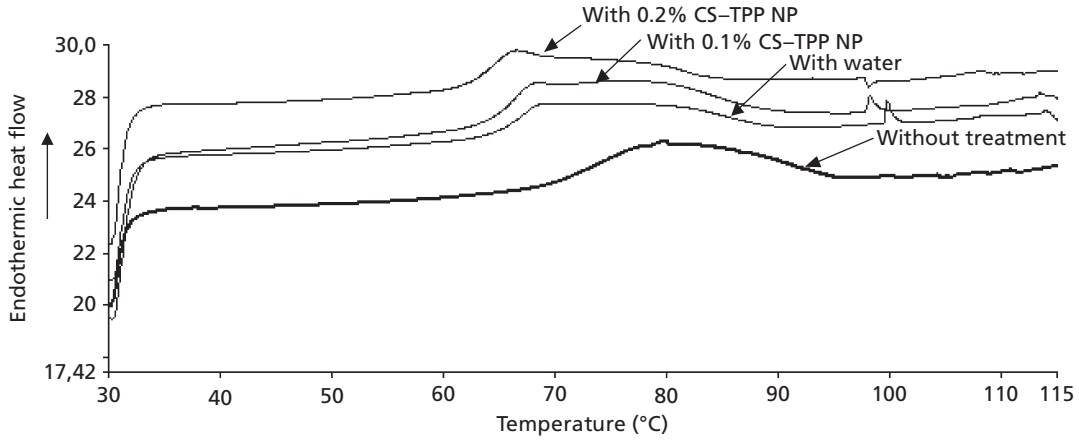


**Figure 7** Thermograms of porcine skin. CS–TPP NP, chitosan–tripolyphosphate nanoparticles.

**Table 4** Thermal changes after impregnation of porcine skin with water, 0.1% or 0.2% chitosan–tripolyphosphate nanoparticles

| Impregnation | Skin–water (control) | Skin–0.1% CS–TPP NP | Skin–0.2% CS–TPP NP |
|---|---|---|---|
| Peak (°C) | 69.82 ± 0.89 | 67.60 ± 0.53 | 66.49 ± 0.28 |
| Onset (°C) | 64.82 ± 0.14 | 63.93 ± 0.31* | 62.09 ± 0.25* |

Values are means ± SD ($n > 4$). CS–TPP NP, chitosan–tripolyphosphate nanoparticles. *$P < 0.05$ vs control (Tukey's test).

the chitosan solution plays a significant role in the formation of nanoparticles.[6] As a consequence of these results, we chose for all further studies nanoparticles produced using chitosan solution at a pH 4.9 that gives optimal PDI and zeta potential values. The chemical stability of aciclovir was improved by incorporation into the nanoparticles, implying successful protection by the nanoparticulate system.

The better permeation of aciclovir from larger nanoparticles could be explained by the interaction of positively charged nanoparticles with anionic components of the epithelial cell surface. The surface charge density of larger nanoparticles may cause a stronger interaction with the cell surface, resulting in better permeation of aciclovir.[21]

Moreover, the DSC measurements indicate a possible interaction between CS–TPP nanoparticles and the skin lipids. Interestingly the larger nanoparticles interacted more strongly, showing lower onset temperature.

## Conclusions

These studies demonstrate that CS–TPP nanoparticles significantly improve the chemical stability of aciclovir, and the possible use of CS–TPP nanoparticles for delivering aciclovir to the skin. Skin diffusion data showed significantly improved permeation of aciclovir from nanoparticles.

The ratio of chitosan to TPP is the most important factor for formation of reproducible nanoparticles. The size of nanoparticles depends on the chitosan content. Increasing the initial chitosan input in the formulation resulted in a larger mean particle diameter. The higher the chitosan content, the higher the aciclovir skin permeation.

## Declarations

### Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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