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RESEARCH PAPER

Development of a Sustained Release Dosage Form for α-Lipoic Acid. I. Design and In Vitro Evaluation

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ABSTRACT

The purpose of this study was the design and evaluation of a sustained release dosage form for the oral administration of α-lipoic acid. The cationic polymer chitosan was used in order to provide a controlled drug release based on ionic interactions with the anionic drug. The effect of such ionic interactions on the release of α-lipoic acid could be verified by diffusion studies. In vitro release studies with tablets (diameter: 10.0 mm; thickness: ~4 mm) containing 80% α-lipoic acid and 20% chitosan acetate showed a controlled drug release over a time period of 24 h. Raising the ratio of chitosan acetate in such delivery systems led to an even stronger retardation of drug release. In addition, permeation studies carried out in Ussing-type chambers with freshly excised intestinal mucosa from guinea pigs demonstrated no significant ($p < 0.05$) influence of the degree of drug ionization on its absorption behavior. The apparent permeability coefficient (P_{app}) for α-lipoic acid was determined to be $1.39 \pm 0.28 \times 10^{-5}$ cm/sec at pH 6.4 (means ± SD). The use of a sustained delivery system for α-lipoic acid, which is based on ionic interactions, should therefore have no influence on the absorption behavior of the drug. The sustained release dosage forms described here might provide a constant plasma level of the drug being highly beneficial for various therapeutic reasons.

Key Words: α-Lipoic acid; Chitosan; Controlled drug release; Oral drug delivery; Permeation studies.

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INTRODUCTION

α-Lipoic acid (thioctic acid) is well known as a potent lipophilic free radical scavenger and naturally occurring antioxidant, which is capable of recycling glutathione and in turn enhancing vitamin E and vitamin C.[1] In parts of Europe it is licensed for the prophylaxis and treatment of polyneuropathy, particularly of diabetic provenience.[2,3] Moreover, in recent years, the drug has additionally gained considerable attention in the treatment of diabetes type II, as it may improve glucose metabolism and insulin secretion via effects on peripheral tissues and the pancreas.[4–6] Although intensive research work has been done in order to explore the underlying molecular mechanisms responsible for all these effects, only minor attempts have been undertaken to develop appropriate oral formulations, which may maximize the therapeutic effect of this drug. Pharmacokinetic studies carried out in humans with conventional α-lipoic acid formulations, for instance, revealed an immediate release spike within 1 hour and a comparatively short elimination half-life ($t_{1/2}$) determined to be 32.7 min for 200 mg and 28.0 min for 600 mg oral dose.[7] On the other hand, a constant plasma level of α-lipoic acid might be highly beneficial for various reasons. Immediate release spikes probably leading to toxic side effects can be avoided (I). The antioxidative and radical scavenging effect might be guaranteed for a prolonged period of time and not just for a few hours (II). In addition, the glucose uptake should be stimulated continually leading to a more pronounced reduction in the blood glucose level (III).

It was therefore the objective of this study to develop a dosage form for α-lipoic acid that can provide such a constant plasma level of the drug. As α-lipoic acid is absorbed from the small intestine and colon to the same extent,[8] the development of a twice or even once daily dosage form seems feasible. In order to guarantee a sustained drug release over such a long period of time, chitosan was chosen as the carrier matrix. Due to ionic interactions between this cationic polymer and the anionic drug—as illustrated in Figure 1—a sustained release of α-lipoic acid from the polymer should be provided. The generation of such a controlled release delivery system from preliminary basic investigations up to first detailed in vitro evaluations will be described within this study.

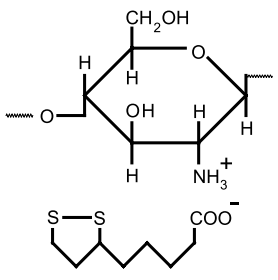

Figure 1. Chemical structure of a chitosan subunit and α-lipoic acid.

MATERIALS AND METHODS

Permeation Studies

Permeation studies were carried out in a slightly modified way as described previously by our research group.[9] In brief, the incubation medium containing 250 mM NaCl, 2.6 mM $MgSO_4$, 10.0 mM KCl, 40.0 mM glucose, and 50 mM $NaHCO_3$ was prepared freshly and buffered with 40 mM Bis–Tris HCl buffer (bis[2-hydroxethyl]aminotris[hydroxymethyl]methane/2-bis[2-hydroxyethyl]amino-2-[hydroxymethyl]-1,3-propanediol hydrochloride; Sigma, St. Louis, MO). The pH was adjusted with 5 M HCl to 5.5, 6.4, and 7.3, respectively. Permeation studies with α-lipoic acid were carried out in Ussing-type chambers displaying a permeation area of 0.64 cm^2. Small intestinal mucosa from the upper part of the ileum of guinea pigs was excised immediately after sacrificing the animal. Without stripping off the underlying muscle layer permeation studies were started within 10 min. All experiments were performed in triplicate in an atmosphere of 95% O_2 and 5% CO_2 at 37° C. After 15–20 min of preincubation with each 1.0 mL of incubation medium in the donor and acceptor chamber, the solution in the donor chamber was substituted by the same medium but containing 0.1% (m/v) α-lipoic acid (SKW Trostberg AG, Trostberg, Germany). For all studies aliquot volumes of 150 μL were withdrawn from the acceptor chamber every 30 min within 3 hours of incubation and substituted by the same medium equilibrated to 37° C. The amount of permeated α-lipoic acid was determined via high-performance liquid chromatography (HPLC) analysis. Samples of 20 μL were directly injected and separated on a C_{18} column (Nucleosil 100-5C18, 150 mm × 3 mm) at 20° C. Elution was performed as follows: flow rate 0.8 mL/min, 0–20 min, eluent 10 mM phosphoric acid/acetonitrile (6:4; v/v). α-Lipoic acid was detected by absorbance at 200 nm with a diode array absorbance detector (Perkin–Elmer 235C, Vienna, Austria). Peak areas were directly proportional to mass of standards injected. Concentrations were determined

by interpolation from a standard curve. Cumulative corrections were made for the previously removed samples in determining the total amount permeated.

Measurement of the Transepithelial Electrical Resistance (TEER)

A Millicell® ERS meter (Millipore Corp., Bedford, MA) connected to a pair of side-by-side electrodes was used to monitor the effect of the polymers on the TEER of the intestinal mucosa. Measurements were performed every 5 min before applying α-lipoic acid and then every 30 min within 3 hours.

Data Analysis

Apparent permeability coefficient (P_{app}) for α-lipoic acid was calculated according to the following equation:

$$P_{app} = Q/A*c*t$$

where P_{app} is the apparent permeability coefficient (cm/s), Q is the total amount permeated throughout the incubation time (μg), A is the diffusion area of the Ussing chamber (cm^2), c is the initial concentration of the drug in the donor compartment ($\mu g/cm^3$), and t is the total time of the experiment (sec).

Diffusion Studies

First, 2.0 g of chitosan (from crab shells, degree of deacetylation >85%; Sigma, St. Louis, MO) were micronized with an ultraturax to a particle size ≤ 2 mm. The polymer was then hydrated in 200 mL of demineralized water for 72 h, and 20 μL, 40 μL, and 80 μL of glacial acetic acid, respectively, were added to aliquot volumes (20 mL) of this suspension. Because of the molar ratio of primary amino groups on chitosan to acetic acid, the resulting salt was called chitosan −1/4 acetate, chitosan-1/2 acetate, and chitosan-1/1 acetate, respectively. Five milliliters of these suspensions, 5.0 mL of the chitosan suspension without acetic acid, and 5.0 mL of demineralized water were each mixed with 5.0 mL of aqueous α-lipoic acid solution being saturated at 10° C. Thereafter, samples were transferred in dialyzing tubings (molecular mass cut-off: 10 kDa), which were placed in beakers containing 40.0 mL of demineralized water. The polymer could not pass this semipermeable membrane, whereas unbound α-lipoic acid was released from the dialyzing tubing. The studies were carried out at 37° C under continuous stirring with a constant exchange area of 40 cm^2 for all tubings. Samples of 0.5 mL were withdrawn from the release medium in hour intervals and substituted by the same volume of demineralized water equilibrated to temperature. The concentration of α-lipoic acid in these samples was determined by HPLC analysis as described above. Cumulative corrections were made for the previously removed samples in determining the total amount diffused.

Preparation of Sustained Release Dosage Forms

First, 5.0 g of chitosan were completely hydrated in 5.0 g (chitosan/acetate 1:1) or in 10.0 g of glacial acetic acid (chitosan/acetate 1:2), respectively, and diluted with demineralized water in order to obtain a final volume of 75 mL. These chitosan/acetate solutions were homogenized with α-lipoic acid in weight ratios as listed in Table 1. After wet granulation through a sieve (mesh: 750 μm), granulates were dried at 37° C and compressed (Korsch, Type EKO–DMS, Berlin, Germany) to tablets (diameter: 10.0 mm; thickness: ~4 mm). The compression force was kept constant during the preparation of all tablets.

Table 1. Composition of chitosan acetate/lipoic acid mixtures for wet granulation followed by compression to tablets (diameter: 10.0 mm; thickness: ~4 mm).

Formulation	Chitosan	Acetic acid	α-Lipoic acid	Demin. H_2O	Determined content (%; m/m) of α-lipoic acid in the resulting dosage form
F100	–	–	100 g	–	100%
F80	13 g	26 g	61 g	167 g	81.4%
F65	19 g	38 g	44 g	240 g	61.4%
F30	28 g	56 g	17 g	361 g	29.7%

The total amount of α-lipoic acid in each formulation was determined via HPLC analysis as described above.

Release Studies

The total load of α-lipoic acid in the tablets described here and from commercially available α-lipoic acid tablets (Thioctacid 300 HR; ASTA Medica AWD GmbH, Frankfurt am Main, Germany) was determined via HPLC analysis. Tablets were grinded and aliquots of 50.0 mg dissolved in 10.0 mL of 50% (v/v) acetic acid for 1 hour at 40° C. Thereafter, 10.0 mL of tetrahydrofuran was added and the dissolution process allowed to proceed for 15 min at 40° C. After the addition of 20.0 mL of demineralized water, aliquots were centrifuged and 20 µL of the supernatant fluid were directly injected for HPLC analysis as described above.

The in vitro release rate of α-lipoic acid from these drug delivery systems was determined by the paddle method according to the European Pharmacopeia. Tablets were placed in the dissolution apparatus filled with 600 mL of either 100 mM Tris–HCl pH 7.5, 100 mM Tris–HCl pH 6.0, or demineralized water. All release studies were performed at 37° C and an agitation of 50 rotations per minute. One-milliliter samples of released α-lipoic acid were withdrawn at predetermined time points and replaced with an equal volume of release medium equilibrated to temperature. The amount of released α-lipoic acid in these samples was determined via HPLC analysis as described above. According standard curves were established for α-lipoic acid in each release medium. Cumulative corrections were made for the previously removed samples in determining the total amount released.

Statistical Data Analysis

Statistical data analysis was performed using the *t*-test with $p<0.05$ as the minimal level of significance.

RESULTS AND DISCUSSION

Permeation Studies

The intestinal absorption of most monocarboxylic acid drugs such as salicylic acid is strongly pH-dependent;[10] i.e., the higher the concentration of the unionized form, the higher the permeability coefficient.

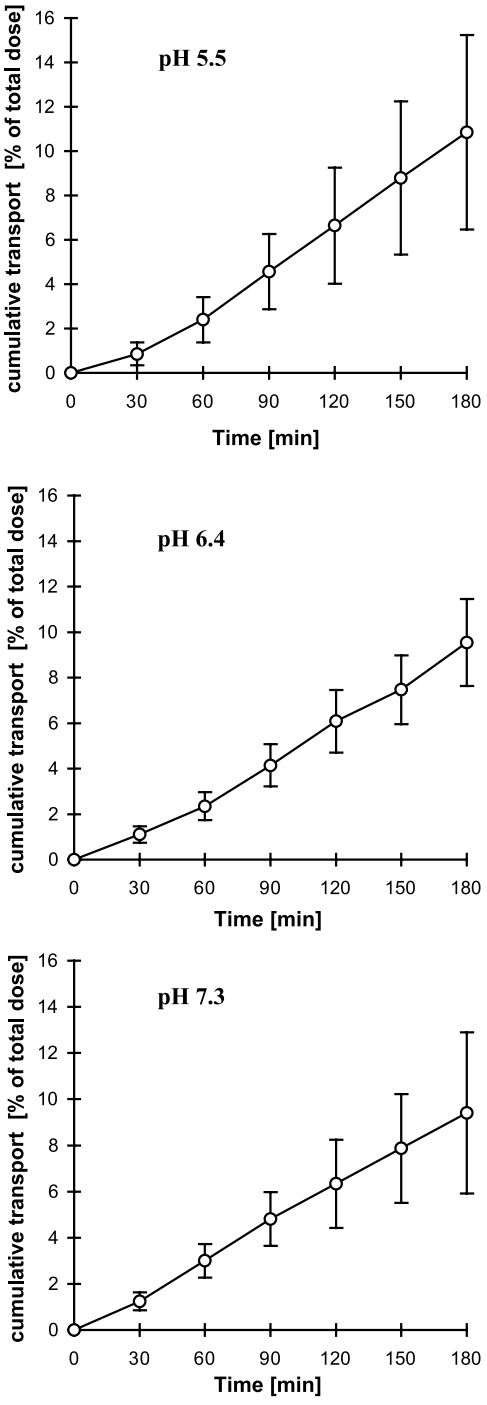

Figure 2. Comparison of the permeation of α-lipoic acid across the intestinal mucosa from guinea pigs. Indicated values are means ± SD of at least three experiments.

Palm et al. could demonstrate a 150- and 30-fold increased transport ratio of the un-ionized form for alfentanil and cimetidine across the absorption membrane, respectively.[11] Regarding the development of an oral delivery system for α-lipoic acid, where a sustained release should be guaranteed by ionic interactions between the drug and the carrier matrix, it was essential to evaluate the influence of the degree of drug ionization on its absorption. Permeation studies, however, demonstrated no significantly different P_{app} values for α-lipoic acid determined to be $1.58 \pm 0.64 \times 10^{-5}$ cm/sec, $1.39 \pm 0.28 \times 10^{-5}$ cm/sec, and $1.37 \pm 0.51 \times 10^{-5}$ cm/sec at pH 5.5, pH 6.4, and pH 7.3, respectively. In permeation studies performed at pH 5.5 the molar ratio of α-lipoic acid to sodium α-lipoate was 45:55, whereas it was 9:91 at pH 6.4 and 1:99 at pH 7.3—calculated on the basis of the pKa value for α-lipoic acid determined to be 5.4.[12] Hence, the permeation of the drug turned out to be completely independent from the degree of drug ionization, which is in contrast to many other monocarboxylic acid drugs displaying a strong pH-dependent absorption behavior.[10] The results of these permeation studies are shown in Figure 2. According to these results, an oral delivery system containing α-lipoic acid in its ionic form should have no negative influence on the intestinal absorption of the drug. Based on these results, the paracellular route of absorption, which depends strongly on the hydrophilicity of the drug-correlating with the degree of drug ionization-, can be excluded as the major pathway across the membrane. These results are in good agreement with previously published results suggesting a carrier-mediated transcellular uptake for monocarboxylic acid drugs.[10] Hence, chitosan, which is well known as a permeation enhancer for the paracellular route of absorption,[13] will not improve the uptake.

Tomita, Hayashi, and Awazu could demonstrate a permeation enhancing effect of sodium caprate for the paracellular route of absorption by a strong decrease in the TEER values.[14] In contrast to their studies, the TEER did not decrease at all due to the addition of α-lipoic acid representing a similar monocarboxylic acid. The results suggest that α-lipoic acid does not display a permeation enhancing effect for the paracellular pathway across the membrane. The constant TEER values throughout all studies partially indicated the viability of the tissue, as in the case of cell damage, in which the TEER would decrease significantly. The viability of the tissue was confirmed by incubating the tissue with trypan blue after each experiment showing no dead cells, which would appear deep blue in the microscope after staining.[9]

Diffusion Studies

In order to guarantee a sustained release of α-lipoic acid from the dosage form over 24 h, chitosan was chosen as the polymeric drug carrier matrix. Based on ionic interactions of this cationic polymer with the anionic drug, α-lipoic acid should be continually released. The results of this study as shown in Figure 3 demonstrated a strong influence of the cationic polymer on the diffusion of the drug. Without chitosan the equilibrium of α-lipoic acid inside and outside the dialyzing tubing was almost reached within 5 h. In contrast, only 63.8% ± 4.3% of the equilibrium concentration was reached when chitosan was added to the donor phase. On the one hand, chitosan can only be hydrated in aqueous solutions in its ionic form.[15] On the other hand, preliminary studies revealed that α-lipoic acid is too hydrophobic as a counter ion to render the polymer swellable. Hence, the addition of a more polar acid was necessary in order to provide the hydratability of the polymer. Because of its comparatively high pKa value determined to be 4.76,[16] which provides in parallel the ionic binding of the drug and excellent hydration of chitosan, acetic acid was chosen. The influence of this acid on the release of α-lipoic acid from chitosan was evaluated by further diffusion studies. The results demonstrated, even at low concentrations of acetic acid, an increase in the retard

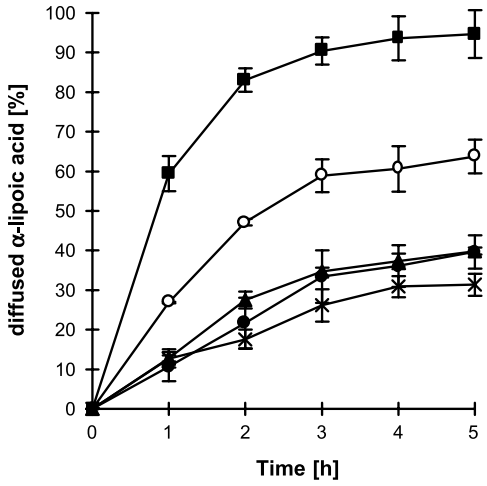

Figure 3. Comparison of the influence of chitosan and acetic acid on the diffusion of α-lipoic acid. Diffusion studies of α-lipoic acid were performed without chitosan (■—■), with chitosan (○—○), with chitosan-1/4 acetate (▲—▲), with chitosan-1/2 acetate (X—X), and with chitosan-1/1 acetate (●—●); values are means ± SD of at least three experiments.

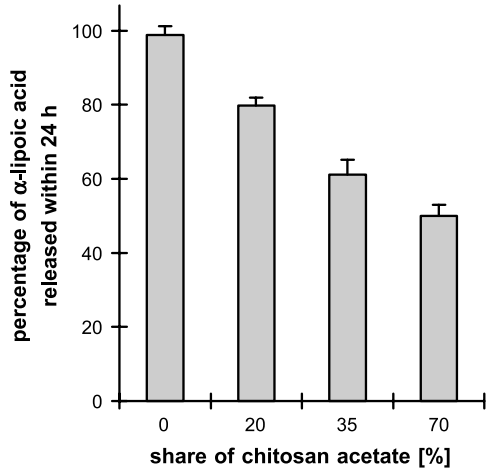

Figure 4. Influence of the ratio of chitosan acetate [%; m/m] in α-lipoic acid tablets on drug release. Values are means ± SD of at least three experiments.

effect of chitosan for the drug; i.e., the ionization of just each fourth primary amino group of chitosan with acetic acid (chitosan-1/4 acetate) led already to a significant reduction in the release rate of α-lipoic acid from the polymer. A reason for this observation can be seen in an increase in free primary amino groups of chitosan, which are accessible to the drug due to the higher degree of polymer hydration mediated by acetic acid. If an acetic acid concentration is reached, which provides the free accessibility of the entire primary amino groups on the polymer for the drug, the retardation of drug release cannot be further enhanced. On the contrary, the further addition of acetic acid seems even to reduce the effect, as the amount of α-lipoic acid diffused out of chitosan-1/1 acetate determined to be 39.8% ± 0.9% within 5 h was significantly higher than that out of the chitosan-1/2 acetate determined to be 31.4% ± 2.8%. This observation can be explained by a competition of the drug and acetic acid for the available amino groups on the polymer. Hence, the more acetic acid is added, the more of the drug should be removed from the polymer. The results of these studies are shown in Figure 3.

Release Studies

Based on the information obtained from simple diffusion studies, according formulations containing α-lipoic acid, chitosan, and acetic acid were generated. As the release behavior of many drugs is strongly pH-dependent,[17] the influence of this parameter was first of all evaluated by according drug release studies carried out at pH 6.0 and 7.5. Studies with chitosan acetate/α-lipoic acid tablets and commercially available oral dosage forms for α-lipoic acid, however, demonstrated no significant influence of the pH and ionic strength on the release behavior. Hence, the following studies were exclusively performed in demineralized water. They demonstrated a clear correlation between the ratio of chitosan in the dosage form and the drug release. The more chitosan was incorporated in the delivery system, the lower was the release rate of α-lipoic acid, from the carrier matrix. The results of this study are shown in Figure 4. They are in good correlation with diffusion studies described above. By the addition of chitosan a sustained drug release seems to be feasible even over a time period of 24 h. The release profile of a commercially available α-lipoic acid tablet and of a tablet based on a wet granulate containing 15 g of chitosan, 15 g of acetic acid, 70 g of α-lipoic acid, and 206 g of demineralized water, for instance, is depicted in Figure 5. It shows an almost zero-order release kinetic for the chitosan containing dosage form within the first 8 h of drug release. Whether this release profile can be maintained under in vivo conditions, however, can only be predicted. Due to ionic interactions with various salts such as bile salts,[18] and because of a partially enzymatic degradation of chitosan in the gastrointestinal (GI)-tract,[19] an at least somewhat accelerated drug release seems likely. Within this study, however, we could demonstrate that an even much stronger retardation of drug release as shown in Figure 5 for a commercially available α-lipoic acid tablet can be achieved by the addition of chitosan. According to these results, an optimization of the in

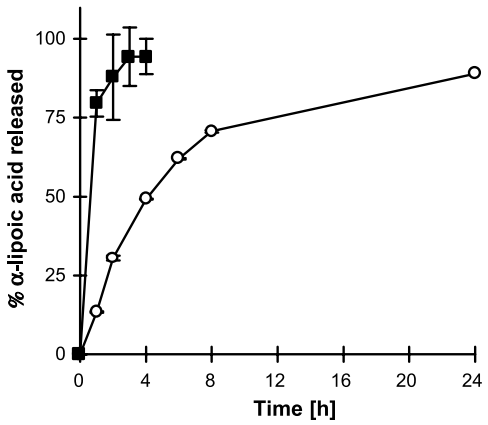

Figure 5. Release profile of commercially available α-lipoic acid tablets (■—■) and α-lipoic acid tablets containing 20% (m/m) chitosan acetate (○—○); indicated values are means ± SD of at least three experiments.

vivo release kinetic should be feasible and is already the subject of ongoing studies.

CONCLUSIONS

The use of chitosan as an auxiliary agent allowed the development of a sustained delivery system for α-lipoic acid. The strong retardation of drug release is based on ionic interactions between the cationic polymer chitosan and the anionic drug, whereas the degree of drug ionization has no influence on its absorption behavior. Chitosan/α-lipoic acid formulations should therefore allow the establishment of once or twice daily dosage forms being highly beneficial for various therapeutic reasons.

ABBREVIATIONS

Bis–Tris:	bis[2-hydroxyethyl]aminotris[hydroxymethyl]methane/2-bis[2-hydroxy ethyl] amino-2-[hydroxymethyl]-1,3-propanediol
P_{app}:	apparent permeability coefficient
TEER:	transepithelial electrical resistance
Tris–HCl:	tris(hydroxymethyl)-aminomethane hydrochloride

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REFERENCES

1. Scott, B.C.; Aruoma, O.I.; Evans, P.J.; O'Neil, C.; Van der Vliet, A.; Cross, C.E.; Tritschler, H.; Halliwell, B. Lipoic and dihydrolipoic acids as antioxidants a critical evaluation. Free Radic. Res. **1994**, *20*, 119–133.
2. Ziegler, D.; Hanefeld, M.; Ruhnau, K.J.; Meissner, H.P.; Lobisch, M.; Schutte, K.; Gries, F.A. Treatment of symptomatic diabetic peripheral neuropathy with the antioxidant α-lipoic acid: a 3-week multicentre randomised controlled trial (ALADIN study). Diabetologia **1995**, *38*, 1425–1433.
3. Ziegler, D.; Mayer, P.; Mühlen, H.; Gries, F.A. Effekte einer Therapie mit α-Liponsäure gegenüber Vitamin B bei der diabetischen Neuropathie. Diabetes Stoffwechs. **1993**, *2*, 443–448.
4. Jacob, S.; Streeper, R.S.; Fogt, D.L.; Hokama, J.Y.; Tritschler, H.J.; Dietze, G.J.; Henriksen, E.J. The antioxidant α-lipoic acid enhances insulin-stimulated glucose metabolism in insulin-resistant rat skeletal muscle. Diabetes **1996**, *45*, 1024–1029.
5. Tsakiridis, T.; McDowell, H.E.; Walker, T.; Downes, C.P.; Hundal, H.S.; Vranic, M.; Klip, A. Multiple roles of phosphatidylinositol 3-kinase in regulation of glucose transport, amino acid transport and glucose transporters in L6 skeletal muscle cells. Endocrinology **1995**, *136*, 4315–4322.
6. Jacob, S.; Henriksen, E.J.; Tritschler, H.J.; Augustin, H.J.; Dietze, G.J. Improvement of insulin-stimulated glucose disposal in type II diabetes after repeated parenteral administration of thioctic acid. Exp. Clin. Endocrinol. Diabetes **1996**, *104*, 284–288.
7. Teichert, J.; Kern, J.; Tritschler, H.-J.; Ulrich, H.; Preiß, R. Investigations on the pharmacokinetics of α-lipoic acid in healthy volunteers. Inter. J. Clin. Pharmacol. Therap. **1998**, *36*, 625–628.
8. Peter, G.; Borbe, H.O. Absorption of [7,8-^{14}C]rac-α-lipoic acid from in situ ligated segments of the gastrointestinal tract of the rat. Arzneim. Forsch. **1995**, *45*, 293–299.
9. Clausen, A.; Bernkop-Schnürch, A. In vitro evaluation of the permeation enhancing effect of thiolated polycarbophil. J. Pharm. Sci. **2000**, *89*, 1253–1261.
10. Takanaga, H.; Tamai, I.; Tsuji, A. pH-Dependent and carrier-mediated transport of salicylic acid across caco-2 cells. J. Pharm. Pharmacol. **1994**, *46*, 567–570.
11. Palm, K.; Luthman, K.; Ros, J.; Grasjo, J.; Artursson, P. Effect of molecular charge on intestinal epithelial drug transport: pH-dependent transport of cationic drugs. J. Pharmacol. Exp. Ther. **1999**, *291*, 435–443.
12. Walton, E.; Wagner, A.F.; Bachelor, F.W.; Peterson, L.H.; Holly, F.W.; Folkers, K. Synthesis of (+)-α-lipoic acid and its optical antipode. J. Am. Chem. Soc. **1955**, *77*, 5144–5148.
13. Lehr, C.-M.; Bouwstra, J.A.; Schacht, H.E.; Junginger, E.H. In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. Int. J. Pharm. **1992**, *78*, 43–48.
14. Tomita, M.; Hayashi, M.; Awazu, S. Comparison of absorption-enhancing effect between sodium

caprate and disodium ethylenediaminetetraacetate in Caco-2 cells. Biol. Pharm. Bull. **1994**, *17*, 753–755.
15. Bernkop-Schnürch, A. Chitosan and its derivatives: potential excipients for peroral peptide delivery systems. Int. J. Pharm. **2000**, *194*, 1–13.
16. Streitwieser, A.; Klein, H.S. Isotope effects on acidity of deuterated formic, acetic, pivalic, and benzoic acids. J. Am. Chem. Soc. **1963**, *85*, 2759–2763.
17. Rupprecht, H. Silicium dioxide and silicates in drug delivery. In *Controlled Drug Delivery*; Müller, B.W., Ed.; APV Band 17 Wiss, Verlags-GesmbH: Stuttgart, 1987; 197–225.
18. *Wissenschaftliche Tabellen Geigy*, 8th Ed.; Ciba-Geigy: Basel, 1983.
19. Nordtveit, R.J.; Varum, K.M.; Smidsrod, O. Degradation of partially N-acetylated chitosans with hen egg white and human lysozyme. Carbohydr. Polym. **1996**, *29*, 163–167.

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