International Journal of Pharmaceutics, 51 (1989) 233–240
Elsevier

IJP 01738

Macromolecular prodrugs. XII. Kinetics of release of naproxen from various polysaccharide ester prodrugs in neutral and alkaline solution

Claus Larsen

Royal Danish School of Pharmacy, Department of Pharmaceutics, Copenhagen (Denmark)

(Received 26 September 1988)
(Accepted 14 October 1988)

Key words: Naproxen–polysaccharide conjugate; Macromolecular ester prodrug; Kinetics of alkaline hydrolysis; Soluble starch conjugate; Hydroxyethyl starch conjugate; Dextran conjugate; Dextran–naproxen glycolic acid ester conjugate

Summary

Naproxen was attached to dextran, soluble starch and hydroxyethyl starch through ester linkages. Drug fixation to dextran was further established by incorporation between the drug and the carrier of a glycolic acid spacer arm. The kinetics of hydrolysis of the naproxen polysaccharide conjugates was studied in aqueous solution over the pH range 6.72–10.53 at 37 °C. The pH dependence of the various pseudo-first-order rate constants showed almost parallel straight-line portions with slopes close to unity, indicating that the hydrolysis reactions were subject to specific base catalysis. The rates of degradation of the conjugates in neutral and alkaline solution differed by a factor of approximately 500 with the naproxen glycolic acid ester derivative as the most sensitive conjugate to undergo base-catalyzed hydrolysis. At physiological pH and temperature, the latter compound degraded with a half-life of 3.6 h. Under the same conditions the half-lives of regeneration of naproxen from the dextran, the soluble starch and the hydroxyethyl starch derivative were 183, 800 and 1600 h, respectively. Although the soluble starch and the hydroxyethyl starch prodrugs possessed excellent sustained release properties in vitro, a major impediment for the applicability of these carrier agents might arise from the limited aqueous solubility of the conjugates even at relatively low drug load. The results obtained have been discussed in relation to design of parenteral depot formulations of NSAID compounds.

Introduction

In recent years, considerable attention has been focused on the use of macromolecular prodrugs in order to improve the delivery characteristics of a wide array of drug compounds (Poznansky and Juliano, 1984; Friend and Pangburn, 1987; Ghose and Blair, 1987; Azori, 1987). A number of polysaccharides have been tested for their usefulness as transport groups for therapeutic agents. Dextran is probably the most prominent member of this group due to excellent physicochemical properties and physiological acceptance (Virnik et al., 1975; Molteni, 1979; Poznansky and Cleland, 1980; Sezaki and Hashida, 1984; Larsen and Johansen, 1985a; Larsen, 1989). Drug conjugates derived from other polysaccharide carriers have been reported, including starch (acetylsalicylic acid

Correspondence: C. Larsen, Royal Danish School of Pharmacy, Department of Pharmaceutics, 2 Universitetsparken, DK 2100 Copenhagen, Denmark.

(Kratzl and Kaufmann, 1961; Kratzl et al., 1961)), inulin (procainamide (Schacht et al., 1984; Remon et al., 1984)), agarose (mitomycin C (Kojima et al., 1978) and adriamycin (Tritton et al., 1983)), soluble starch (nicotinic acid (Puglisi et al., 1976) and salicylates (Havron et al., 1974)), cellulose (metribuzin (McCormick and Lichatowich, 1979) and insulin (Singh et al., 1981)) and hydroxypropyl cellulose (estrone and testosterone (Yolles, 1978; Yolles et al., 1979)). Drug attachment to polysaccharide carriers has been established through a variety of chemical bond types. The majority of the synthesized high molecular weight derivatives have been proposed to act in a prodrug manner exhibiting sustained release properties. However, only in few cases were the regeneration rates of the drug from the respective conjugates determined.

The stability of bioreversible dextran ester conjugates of benzoic acids (Larsen and Johansen, 1985b; Larsen et al., 1986) and NSAID compounds (Harboe et al., 1988; Larsen and Johansen, 1989) has been studied in detail. The present study was undertaken in order to provide basic information about the release pattern of drugs from other polysaccharide ester conjugates, using naproxen as model drug compound.

Materials and Methods

Dextran T-70 (M_w 74,300; M_n 36,000) and T-500 (M_w 488,000; M_n 184,800) were obtained from Pharmacia, Sweden. Hydroxyethyl starch and naproxen were purchased from Sigma, U.S.A. The soluble starch sample employed was a Ph. Eur. quality. (2-((+)-2-(6-methoxy-2-naphthyl)propionyloxy)acetic acid (naproxen glycolic acid ester) was synthesized by reacting the acid chloride of naproxen with glycolic acid, m.p. 123–125 °C. Naproxen and the corresponding glycolic acid ester were linked to the polysaccharides by using *N,N'*-dicyclohexylcarbodiimide as condensing agent (Harboe et al., 1988). The conjugates were characterized as previously described (Larsen and Johansen, 1985b; Johansen and Larsen, 1985). The degree of substitution (DS) has been expressed as the percentage of mg naproxen released per mg of the conjugate. Acetonitrile used in the HPLC procedures was of chromatographic grade. Buffer substances and all other chemicals were of an analytical or a reagent grade.

Apparatus

HPLC analysis of the dextran and hydroxyethyl starch ester prodrugs was performed with system A: a Waters Ass. Model 6000A constant-flow pump, a Waters Ass. Model 450 variable wavelength detector and a Rheodyne Model 7125 injection valve with a 20 μl loop. The column, 50 × 8 mm, was packed with spherically shaped Nucleosil Diol 7-OH particles (7 μm) (Mackerey-Nagel, F.R.G.). The soluble starch–naproxen conjugate was chromatographed, using the HPLC system B, consisting of a Hitachi Model 655A-11 solvent delivery pump equipped with a variable wavelength Hitachi F1000 fluorescence detector, a Rheodyne Model 7125 injection valve with a 20 μl loop and a Hitachi Model D2000 chromato-integrator. A Nucleosil Diol column, 250 × 8 mm, was used. During chromatography the column was protected by a small pre-column packed with Nucleosil Diol and by a silica saturation column positioned between the pump and the injection valve. The latter column was packed with LiChroprep SI 60 15–25 μm (Merck, F.R.G.). Ultraviolet spectral measurements were carried out using a Shimadzu UV-190 spectrophotometer. Readings of pH were done on a Radiometer Type pH M26 meter. Melting point was taken on a capillary melting-point apparatus and is uncorrected.

Kinetic measurements

The rates of hydrolysis of the polysaccharide–naproxen ester prodrugs were determined in aqueous buffer solutions at a constant temperature of 37 ± 0.2 °C. Phosphate, borate and carbonate buffers were employed. Each buffer was adjusted to an ionic strength of 0.5 by addition of a calculated amount of potassium chloride. Pseudo-first-order rate constants were derived from the slopes of linear plots of ln p_t versus time, where p_t refers to the peak height/area of intact conjugate at time t. The reactions were initiated by adding the conjugates to the pre-

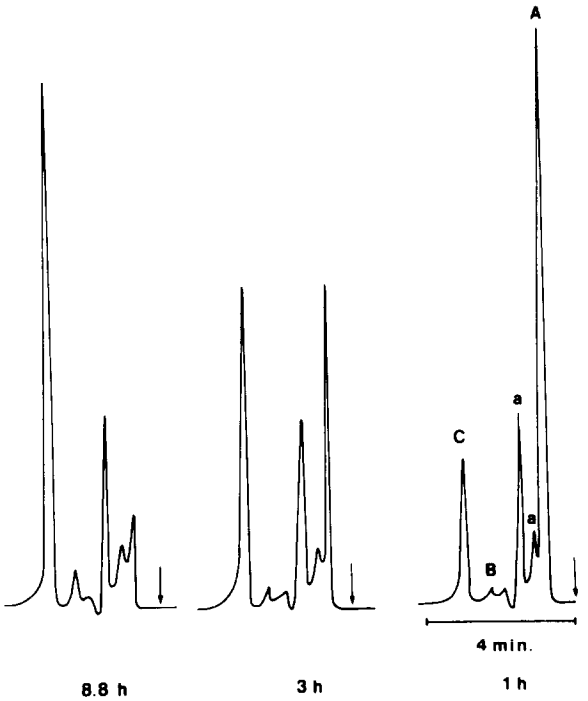
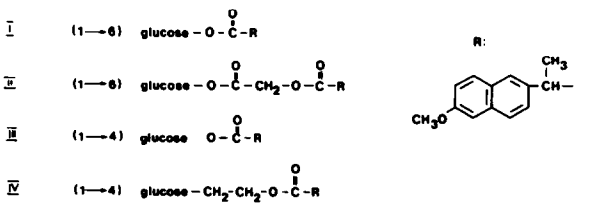


Fig. 1. Hydrolysis of a dextran T-500–naproxen glycolic acid ester conjugate (A) (DS 0.7) in 0.05 M phosphate buffer pH 7.50 (37 °C) as followed by HPLC system A. 20 μl samples were chromatographed at the times indicated. (B): naproxen; (C): naproxen–glycolic acid ester; a: unidentified peaks.

heated buffer solutions: dextran T-70 (I) (1.6 mg · ml^{-1}), dextran T-500 glycolic acid ester (II) (1.0 mg · ml^{-1}) and hydroxyethyl starch (IV) (0.5 mg · ml^{-1}). The reaction mixtures were analyzed, by using HPLC system A, for intact conjugate. In case of the high molecular weight naproxen–glycolic acid ester derivative also the released low molecular weight products were monitored. By employing a mobile phase composed of 0.05 M phosphoric acid–acetonitrile (7 : 3 v/v) at a flow rate of 1.2 ml · min^{-1} separation of the individual compounds was achieved within 4 min (Fig. 1). The eluated compounds were monitored at 271 nm. The soluble starch–naproxen derivative was sparingly soluble in aqueous solution and dissolved only upon heat treatment (100 °C for 5 min). Therefore a stock solution was prepared in water. Aliquots of the latter solution were added to the appropriate buffer to give an initial concentration corresponding to 100 μg · ml^{-1}. HPLC system B was used for the analysis of the conjugate with the wavelengths being $\lambda_{ex} = 330$ nm and $\lambda_{em} = 360$ nm. The eluent was composed of 0.05 M phosphoric acid–acetonitrile (9 : 1 v/v). Quantitation of the respective entities was done from peak height/area measurements in relation to those of standards chromatographed under the same conditions.

Results and Discussion

The kinetics of hydrolysis of the various polysaccharide esters of naproxen (**I–IV**) were studied in aqueous buffer solution in the pH range 6.72–10.51 at 37 °C. Under the experimental conditions employed, the decomposition reactions displayed first-order kinetics. The concentration of the buffers used (0.05 M) had no significant influence on the hydrolysis rates.



The pH dependence of the pseudo-first-order rate constant, k_{obs}, is shown in Fig. 2 and Table 1. The pH–rate profiles exhibited straight-line portions for pH above 6.7 with slopes close to unity (α ranging from 0.83 to 1.06). Thus, in the investigated pH range, the rates of hydrolysis were proportional to the hydroxide ion activity, a_{OH}, in accordance with the following rate expression:

$$k_{obs} = k_{OH} a_{OH} \quad (1)$$

where k_{OH} is the second-order rate constant for specific base-catalyzed hydrolysis. a_{OH} was calculated according to Harned and Hamer (1933):

$$\log a_{OH} = \mathrm{pH} - 13.62 \quad (2)$$

The values of k_{OH} for the different naproxen conjugates are listed in Table 2. The obtained value for the dextran ester prodrug (compound **I**) is in close agreement with the one of 90 M^{-1} ·

TABLE 1

Pseudo-first-order rate constants, k_{obs}, for the hydrolysis of various polysaccharide–naproxen ester conjugates in aqueous solution in the pH range 6.72–10.51 at 37 °C and an ionic strength of 0.5

Naproxen conjugate	k_{obs} (h^{-1})						
	pH 6.72	pH 7.38	pH 8.78	pH 9.10	pH 9.53	pH 10.01	pH 10.51
Dextran T-70 (**I**)	–	3.8×10^{-3}	7.2×10^{-2}	1.8×10^{-1}	–	1.3	–
Dextran T-500 glycolic acid ester (**II**)	4.7×10^{-2}	1.9×10^{-1}	2.7	4.4	–	–	–
Hydroxyethyl starch (**IV**)	–	–	–	2.0×10^{-2}	–	1.4×10^{-1}	3.6×10^{-1}
Soluble starch (**III**)	–	–	–	–	9.8×10^{-2}	3.0×10^{-1}	1.1

min^{-1} previously reported (calculated from measurements of the initial rates of product formation (Larsen and Johansen, 1989)). Interestingly, the naproxen-soluble starch ester bond (compound **III**) is almost 5 times less susceptible to alkaline hydrolysis than is the dextran–drug ester linkage. In both cases naproxen is covalently attached to monomeric glucose unit hydroxy groups. Dextran is composed of (1 → 6)-linked α-D-glucopyranosyl residues. The pK_a value of the carbohydrate hydroxy group is rather low (11.78 at 37 °C) (Larsen, to be published) most likely due to the stabilization of the anion by intramolecular hydrogen-bonding effected by the adjacent hydroxy groups (Haines, 1976). In contrast, starch is built up of glucose units connected by 1,4-bonds. A possible explanation for the observed difference in hydrolytic reactivity of the two carrier conjugates might therefore be that the structure of starch allows only less energetically favoured anion complexes to be formed, which in turn might result in less acidic hydroxy groups. This hypothesis appears conceivable from the reported differences in pK_a values of various polyhydroxy compounds: cyclohexaamylose (12.3 at 30 °C (Gelb et al., 1980); van Etten et al., 1967)), glucose (12.4 at 25 °C (Beenackers et al., 1985)), mannitol and sorbitol (13.50 and 13.56 at 18 °C (Thamsen, 1952)) and adenosine (12.5 at 25 °C (Izatt et al., 1965)).

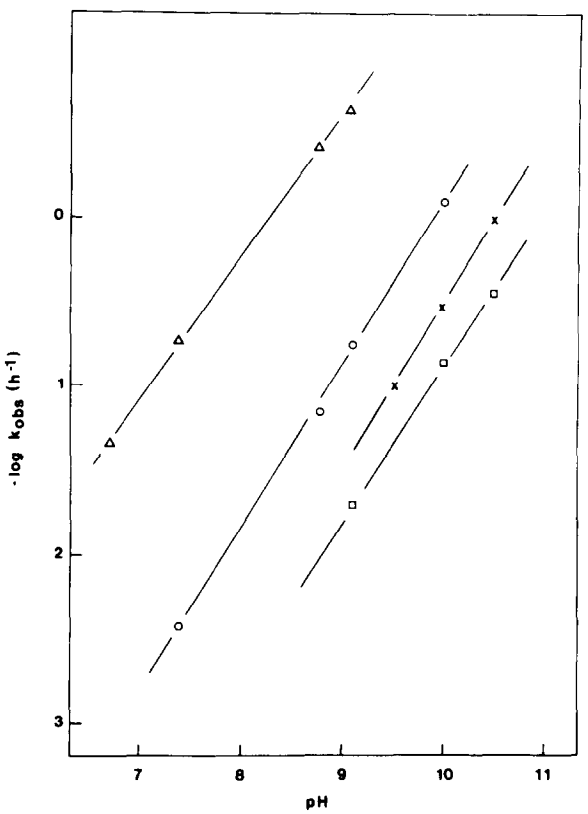


Fig. 2. pH–rate profiles for hydrolysis of: ○, a dextran T 70–naproxen ester conjugate (**I**) (DS 5.6); △, a dextran T 500–naproxen glycolic acid ester conjugate (**II**) (DS 0.7) (×): a soluble starch-naproxen ester conjugate (**III**) (DS 0.8); and □, a hydroxyethyl starch–naproxen ester conjugate (**IV**) (DS 1.6) at 37 °C and $\mu = 0.5$.

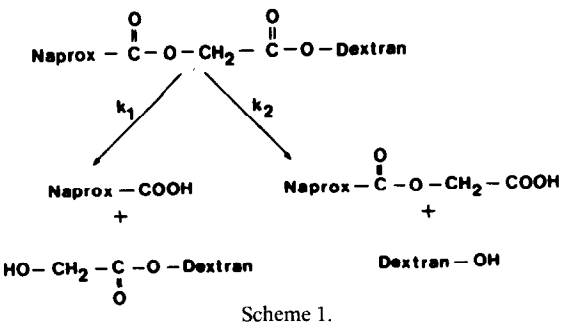
TABLE 2

Specific base catalytic rate constants, k_{OH}, (in $M^{-1} \cdot min^{-1}$) for the degradation of the polysaccharide-naproxen ester compounds **I–IV** *at 37 °C and $\mu = 0.5$*

	I	**II**	**III**	**IV**
k_{OH}	95	4500	21	9.4

Hydroxyethyl starch (HES) is in clinical use as a synthetic colloid plasma volume expander (Köhler et al., 1982; Yacobi et al., 1982). The degree of substitution of therapeutic HES is about 0.7 (70 hydroxyethyl groups per 100 glucose units) in order to avoid α-amylase facilitated depolymerization of the starch backbone (Mishler et al., 1977; Mishler et al., 1979). The stabilities of compound **III** and the naproxen–hydroxyethyl starch derivative (compound **IV**) differ only by a factor of 2. Although the DS of the purchased HES sample was not specified, the data strongly indicate that naproxen is attached to the starch hydroxy groups as well as the terminal OH-group of the hydroxyethyl spacer arm, assuming a pK_a value of the latter alcoholic group of about 16 (Ballinger and Long, 1960). In a study of the hydrolysis of benzoic acid esters derived from dextran and ethanol a ratio k_{OH} (dextran ester)/k_{OH} (ethanol ester) of 15 was found (Larsen and Johansen, 1985b). The actual location of naproxen within the HES molecule might be a consequence of the reaction conditions or be due to the tendency of polysaccharide esters to undergo acyl migration (Garegg, 1965; Reicher and Correa, 1984; Kamerling et al., 1987) through intermediate ortho acid ester formation (Casinovi et al., 1974). This common feature of the ester derivatives has lead de Belder and Norrman (1968) to suggest that the distribution of substituents is thermodynamically rather than kinetically controlled.

Incorporation of a glycolic acid spacer arm between naproxen and the dextran matrix leads to a conjugate (compound II) endowed an enhanced lability in alkaline solution in proportion to that of compound **I**. As seen from Scheme 1 compound **II** possesses two ester functions susceptible to undergo hydrolytic disruption. The hydrolysis of the latter derivative was found to proceed by parallel formation of the parent drug and the naproxen–glycolic acid ester (represented by the first-order rate constants k_1 and k_2, respectively). From Fig. 2, showing the time-courses for the various species in the decomposition of compound **II**, it is apparent that formation of the naproxen–glycolic acid ester greatly exceeds that of the drug per se. A ratio k_1/k_2 of 0.053 has been calculated.



Scheme 1.

The corresponding ratio for hydrolysis methyl *O*-benzoylglycolate amounted to 0.087 (Nielsen and Bundgaard, 1987). The dextran alkoxide ion is a better leaving group compared to the methoxide ion, and this feature probably accounts for the observed difference in product formation ratios. Since esterification of the glycolic acid OH-group by naproxen renders the free acid group more acid, the k_{OH} (compound **II**)/k_{OH} (compound **I**), corresponding to approximately 50, might be explained in terms of a lower pK_a value of the latter derivative compared to that of parent naproxen (4.15 (Fini et al., 1985); 4.20 (Larsen and Johansen, 1989)). In support of this suggestion, data for the alkaline hydrolysis of *p*-substituted benzoic acid esters of dextran are reported, revealing that

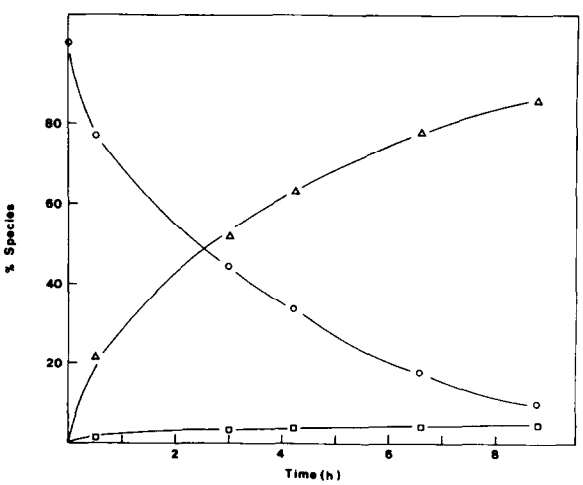


Fig. 3. Time courses for dextran T-500–naproxen–glycolic acid ester (**II**) (○), naproxen–glycolic acid ester (△) and naproxen (□) during the hydrolysis of (**II**) in 0.05 M phosphate buffer pH 7.50 at 37 °C ($\mu = 0.5$).

base-catalyzed hydrolysis of the dextran ester bond is facilitated by low electron density at the reaction site (Larsen et al., 1986).

The accomplishment of localized and prolonged duration of drug action after intravenous administration of macromolecular prodrugs is, in general questionable. At least two major reasons may account for this: (i) the multitude of physiological barriers that the carrier conjugate has to fight against on its way from the administration site to the ultimate target of the drug entity (Poznansky and Juliano, 1984; Gardner, 1985); and (ii) the task of achieving the correct timing of the events leading to optimal drug action, e.g. selective regeneration and suitable maintenance of the active agent at the pharmacological site (Tomlinson, 1987; Stella and Himmelstein, 1980). However, by exploitation of the restricted in vivo mobility of high molecular weight polysaccharide carriers, local drug release may occur after injection of the prodrug in the vicinity of the diseased site. For example, after intratumoral injection in mice of a dextran–mitomycin C prodrug, the conjugate showed a superior effect on subcutaneously implanted B-16 melanoma compared to the parent drug (Hashida et al., 1981). In addition, this approach appears attractive to provide intra-articular depot formulations of NSAID compounds (Larsen and Johansen, 1989).

In the latter connection, the naproxen–glycolic acid ester–dextran conjugate (compound **II**) is of little value. At physiological pH it degrades rapidly with a half-life of 3.6 h. Furthermore, the released naproxen–glycolic acid ester derivative is presumably not readily converted to the NSAID compound within the joint. This being based on observations which show that the corresponding benzoic acid and indomethacin derivatives are poor substrates for enzymes (Nielsen and Bundgaard, 1987; Dell et al., 1980). The naproxen ester conjugates of soluble starch and hydroxyethyl starch possess excellent sustained release properties in vitro with estimated half-lives at pH 7.4 of about 800 and 1600 h, respectively. A major impediment for the applicability of these carrier agents may arise from the limited aqueous solubility of the conjugates even at relatively low drug load. Injection of suspensions of the derivatives into an inflamed joint cavity is not to be recommended due to the risk of appearance of local flare reaction provoked by the physical nature of such microparticles (Hunneyball, 1986). As regards the potential utility of the investigated polysaccharide derivatives to provide intra-articular depot formulations of NSAIDs, it can therefore be concluded that the dextran prodrug with the nonsteroidal anti-inflammatory agent linked directly to the carrier matrix may represent the most promising parenteral drug delivery system.

References

Azori, M., Polymeric prodrugs. *CRC Critical Reviews in Therapeutic Drug Carrier Systems*, 4 (1987) 39–65.

Ballinger, P. and Long, F.A., Acid ionization constants of alcohols II. Acidities of some substituted methanols and related compounds. *J. Am. Chem. Soc.*, 82 (1960) 795–798.

Beenackers, J.A.W.M., Kuster, B.M. and van der Baan, H.S., Ionisation and solvation of D-glucose. *Carbohydr. Res.*, 140 (1985) 169–183.

Casinovi, C.G., Framondino, M., Randazzo, G. and Siami, F., Acetyl migration in the monoacetates of methyl 6-O-trityl-α-D-glucopyranoside. *Carbohydr. Res.*, 36 (1974) 67–73.

Dell, H.-D., Doersing, M., Fischer, W., Jacobi, H., Kamp, R., Köhler, G. and Schöllnhammer, G., Metabolismus und Pharmakokinetik von Acemetacin beim Menschen. *Arzneim.-Forsch.*, 30 (1980) 1391–1398.

de Belder, A.N. and Norrman, B., The distribution of substituents in partially acetylated dextran. *Carbohydr. Res.*, 8 (1968) 1–6.

Fini, A., Zecchi, V. and Tartarini, A., Dissolution profiles of NSAID carboxylic acids and their salts with different counter ions. *Pharm. Acta Helv.*, 60 (1985) 58–62.

Friend, D.R. and Pangburn, S., Site-specific drug delivery. *Med. Res. Rev.*, 7 (1987) 53–106.

Gardner, C.R., Potential and limitations of drug targeting. *Biomaterials*, 6 (1985) 153–160.

Garegg, P.J., Migration of an acetyl group between the C-2 and C-3 hydroxyl groups in methyl mannopyranosides. *Arkiv Kemi*, 23 (1965) 255–268.

Gelb, R.I., Schwartz, L.M., Bradshaw, J.J. and Laufer, D.A., Acid dissociation of cyclohexaamylose and cycloheptaamylose. *Bioorg. Chem.*, 9 (1980) 299–304.

Ghose, T.I. and Blair, A.H., The design of cytotoxic-agent-antibody conjugates. *CRC Critical Reviews in Therapeutic Drug Carrier Systems*, 3 (1987) 262–361.

Haines, A.H., Relative reactivities of hydroxy groups in carbohydrates. *Adv. Carbohydr. Chem. Biochem.*, 33 (1976) 11–109.

Harboe, E., Johansen, M. and Larsen, C., Macromolecular prodrugs VI. Coupling of the highly lipophilic agent

naproxen to dextrans and in vitro characterization of the conjugates. *Farmaci, Sci. Ed.* 16 (1988) 73–85.

Harned, H.S. and Hamer, W.J., The ionisation constant of water in potassium chloride solutions from electromotive forces of cells without liquid junction. *J. Am. Chem. Soc.*, 55 (1933) 2194–2206.

Hashida, M., Kato, A., Kojima, T., Muranishi, S., Sezaki, H., Tanigawa, N., Satomura, K. and Hikasa, Y., Antitumor activity of mitomycin C–dextran conjugate against various murine tumors. *Gann*, 72 (1981) 226–234.

Havron, A., Weiner, B.-Z. and Zilkha, A., Polymeric salicylate derivatives. *J. Med. Chem.*, 17 (1974) 770–772.

Hunneyball, I.M., Intra-articular administration of drugs. *Pharm. Int.*, (1986) 118–122.

Izatt, R.M., Hansen, L.D., Rytting, J.H. and Christensen, J.J., Proton ionisation of adenosine. *J. Am. Chem. Soc.*, 87 (1965) 2760–2761.

Johansen, M. and Larsen, C., Macromolecular prodrugs II. Influence of variation in molecular weight and degree of substitution of *O*-benzoyl–dextran conjugates on their physicochemical properties and stability in aqueous buffer and in plasma. *Int. J. Pharm.*, 27 (1985) 219–231.

Kamerling, J.P., Schauer, R., Shukla, A.K., Stoll, S., Halbeek, H.V. and Vliegenthart, F.G., Migration of *O*-acetyl groups in *N*,*O*-acetylneuraminic acids. *Eur. J. Biochem.*, 162 (1987) 601–607.

Köhler, H., Zschiedrich, H., Linfante, A., Appel, F., Pitz, H. and Clasen, R., Die Elimination von Hydroxyäthylstärke 200/0.5, Dextran 40 und Oxypolygelatine. *Klin. Wochenschr.*, 60 (1982) 292–301.

Kojima, T., Hashida, M., Muranishi, S. and Sezaki, H., Antitumor activity of timed-release derivative of mitomycin C, agarose bead conjugate. *Chem. Pharm. Bull.*, 26 (1978) 1818–1824.

Kratzl, K. and Kaufmann, E., Versuche zur Darstellung hochmolekularer Pharmazeutika. 1 Mitt.: Synthese, Reaktionen und ^{14}C-Markierung eines Acetylsalicylsäure stärkeesters. *Monatschr. Chem.*, 92 (1961) 371–378.

Kratzl, K., Kaufmann, E., Kraupp, O. and Stormann, H., Versuche zur Darstellung hochmolekularer Pharmazeutika, 2. Mitt.: Stoffwechseluntersuchungen von Acetylsalicylsäurestärkeestern. *Monatschr. Chem.*, 92 (1961) 378–383.

Larsen, C., Dextran prodrugs – structure and stability in relation to therapeutic activity. *Adv. Drug Delivery Rev.*, (1989) in press.

Larsen, C. and Johansen, M., Dextrans as carriers for drug compounds—realized and potential applications. *Arch. Pharm. Chemi*, 92 (1985a) 809–830.

Larsen, C. and Johansen, M., Macromolecular prodrugs I. Kinetics and mechanism of hydrolysis of *O*-benzoyl dextran conjugates in aqueous buffer and in human plasma. *Int. J. Pharm.*, 27 (1985b) 205–218.

Larsen, C. and Johansen, M., Macromolecular prodrug XI. Regeneration rates of various NSAID compounds from their corresponding dextran ester prodrugs in aqueous buffer and in different biological media. *Acta Pharm. Nord.*, in press.

Larsen, C., Johansen, M. and Harboe, E., Macromolecular prodrugs III. Linear free energy relationship for hydrolysis of various *para*-substituted benzoate esters of dextran in neutral and alkaline solution. *Arch. Pharm. Chemi., Sci. Ed.* 14 (1986) 44–51.

McCormick, C.L. and Lichatowich, D.K., Homogeneous solution reactions of cellulose, chitin and other polysaccharides to produce controlled-activity pesticide systems. *J. Polym. Sci. Polym. Lett. Ed.*, 17 (1979) 479–484.

Mishler, J.M., Borberg, H., Emerson, P.M. and Gross, R., Hydroxyethyl starch: an agent for hypovolemic shock treatment. *J. Surg. Res.*, 23 (1977) 239–245.

Mishler, J.M., Ricketts, C.R., Parkhouse, E.J., Borberg, H. and Gross, R., Catabolism of low-molecular weight hydroxyethylated amylopectin in man. *J. Lab. Clin Med.*, 94 (1979) 841–847.

Molteni, L., Dextrans as drug carriers. In Gregoriadis, G. (Ed.), *Drug Carriers in Biology and Medicine*, Academic, London, 1979, pp. 107–125.

Nielsen, N.M. and Bundgaard, H., Prodrugs as drug delivery systems. 68. Chemical and plasma-catalyzed hydrolysis of various esters of benzoic acid: a reference system for designing prodrug esters of carboxylic acid agents. *Int. J. Pharm.*, 39 (1987) 75–85.

Poznansky, M.J. and Cleland, L.G., Biological macromolecules as carriers of drugs and enzymes. In Juliano, R.L. (Ed.), *Drug Delivery Systems*, Oxford University Press, New York, 1980, pp. 253–320.

Poznansky, M.J. and Juliano, R.L., Biological approaches to the controlled delivery of drugs: a critical review. *Pharmacol. Rev.*, 36 (1984) 277–343.

Puglisi, L., Caruso, V., Paoletti, R., Ferruti, P. and Tanzi, M.C., Macromolecular drugs I: Long-lasting antilipolytic activity of nicotinic acid bound to a polymer. *Pharmacol. Res. Commun.*, 8 (1976) 379–386.

Reicher, F. and Correa, J.B.C., Location of *O*-acetyl groups in the acidic D-xylan of mimosa scabrella (bracatinga). A study of *O*-acetyl group migration. *Carbohydr. Res.*, 135 (1984) 129–140.

Remon, J.P., Duncan, R. and Schacht, E., Polymer-drug combinations: pinocytic uptake of modified polysaccharides containing procainamide moieties by rat visceral yolk sacs cultured in vitro. *J. Controlled Release*, 1 (1984) 47–56.

Schacht, E., Ruys, E., Vermeersch, J. and Remon, J.P., Polymer-drug combinations: synthesis and characterization of modified polysaccharides containing procainamide moieties. *J. Controlled Release*, 1 (1984) 33–46.

Sezaki, H. and Hashida, M., Macromolecule–drug conjugates in targeted cancer chemotherapy. *CRC Critical Reviews in Therapeutic Drug Carrier Systems*, 1 (1984) 1–38.

Singh, M., Vasudevan, P., Sinha, T.J.M., Ray, A.R., Misro, M.M. and Guha, K., An insulin delivery system from oxidized cellulose. *J. Biomed. Mater. Res.*, 15 (1981) 655–661.

Stella, V. and Himmelstein, K.J., Prodrugs and site-specific drug delivery. *J. Med. Chem.*, 23 (1980) 1275–1282.

Thamsen, J., The acidic dissociation constants of glucose,

mannitol and sorbitol, as measured by means of the hydrogen electrode and the glass electrode at 0 and 18 °C. *Acta Chem. Scand.*, 6 (1952) 270–284.

Tomlinson, E., Theory and practice of site-specific drug delivery. *Adv. Drug Delivery Rev.*, 1 (1987) 87–198.

Tritton, T.R., Yee, G. and Wingaard, Jr., L.B., Immobilized adriamycin: a tool for separating cell surface from intracellular mechanisms. *Fed. Proc.*, 42 (1983) 284–287.

Van Etten, R.L., Clowes, G.A., Sebastian, J.F. and Bender, M.L., The mechanism of the cycloamylose-accelerated cleavage of phenyl esters. *J. Am. Chem. Soc.*, 89 (1967) 3253–3262.

Virnik, A.D., Khomyakov, K.P. and Skokova, I.F., Dextrans and its derivatives. *Russian Chem. Rev.*, 7 (1975) 1280–1307.

Yacobi, A., Stoll, R.G., Sum, C.Y., Lai, C.-M., Gupta, S.D. and Hulse, J.D., Pharmacokinetics of hydroxyethyl starch in normal subjects. *J. Clin. Pharmacol.*, 22 (1982) 206–212.

Yolles, S., Time-release depot for anticancer drugs: release of drugs covalently bonded to polymers. *J. Parenteral Drug Ass.*, 32 (1978) 188–191.

Yolles, S., Morton, J.F. and Sartori, M.F., Preparation of steroid esters of hydroxypropyl cellulose. *J. Polym. Sci. Polym. Chem. Ed.*, 17 (1979) 4111–4113.