International Journal of Pharmaceutics, 30 (1986) 133–141
Elsevier

IJP 01004

Characterization and evaluation of dialdehyde starch as an erodible medical polymer and a drug carrier

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(Received September 13th, 1985)
(Modified version received December 2nd, 1985)
(Accepted December 17th, 1985)

Key words: dialdehyde starch – drug carrier – dissolution – degradation – polymer

Summary

The characteristics of dialdehyde starch as a polymer, particularly molecular weight distribution, dissolution and degradation behavior were investigated. The effects of salts, pH and temperature on dissolution and degradation were studied. Moreover, the usefulness of dialdehyde starch as a drug carrier was evaluated in vitro using its conjugate with isoniazid.

Introduction

A great amount of chemical research on dialdehyde starch (DAS) and the conjugates, with amino-compounds and hydrazino-compounds, has been carried out (Barry and Mitchell, 1953; Barry and Mitchell, 1954; Mester, 1955). DAS, having the aldehyde group as a reactive group, can be utilized as an urea adsorption agent for the medical treatment of chronic kidney disease and uremia (Shimizu et al., 1982). The particles of activated oxidized polysaccharide coated with such large molecules as albumins, gelatins and polyvinyl alcohols were invented in order to improve these characteristics (Shimizu et al., 1982a and b). Although the degradation products were investigated under alkaline conditions by Whistler et al. (1959), the dissolution and the degradation rate, which were very important medically and pharmaceutically, have not been studied thoroughly. Therefore, these properties were investigated in this work.

The incorporation of a biologically active agent into a polymer and a sustained release drug delivery system have been important pharmaceutical subjects. Many polymers have been utilized but various problems with immunotoxicities and accumulation in organs have occurred frequently for parenteral administration. DAS may be regarded as being respectably relieved of the above defects. It was reported that the conjugate between DAS and isoniazid (INAH) or thiosemicarbazide strongly inhibited the growth of *Mycobacterium tuberculosis* (Barry and Mitchell, 1953). Then in this study, the in vitro property of the conjugate between DAS and a drug, created by hydrazone-bonding, is reported.

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Materials and Methods

Materials

The following materials were used; 50% oxidized starch or dialdehyde starch (DAS-Q50) from the Japan Carlit Co. (Watanobe and Shibuya, 1966); isoniazid (INAH) and isonicotinic acid (INA) from the Wako Pure Chemical Industries and isonicotinamide (INAA) from the Aldrich Chemical Co.

Preparation of the conjugate between DAS-Q50 and INAH

DAS-Q50 (5.4 g) was suspended in 100 ml of deionized water and INAH (4.6 g) was added to the suspension. Then, pH was adjusted to 4.3–4.4 by acetic acid. After stirring for 20 h, the suspension was filtered by a glass filter and the pale yellow precipitate was collected. It was washed quickly with a 50% acetone aqueous solution and dried in a vacuum desiccator at room temperature for 2 days. The product was named DAS-Q50–INAH.

Characterization of DAS-Q50 and DAS-Q50–INAH as powders

The crystallinity of DAS-Q50 and DAS-Q50–INAH was investigated by powder X-ray diffractometry, using a Rigaku Denki Geigerflex Model D2 diffractometer with Ni-filtered Cu-Kα radiation. Further, DAS-Q50 maximum particle dimensions were measured for 150 particles picked at random, using a JEOL JSM T200 scanning electron microscope where the sample was coated with gold layer (about 200 Å thick).

Estimation of DAS-Q50 degradation behavior

Two Sephadex G100 columns were prepared. One (1.4 × 22.0 cm) was used for a 1/15 M phosphate buffer, pH 7.4 as an elution solvent and the other (1.4 × 24.5 cm) for a 0.5% CH_3COONa aqueous solution, pH 7.2. The gel-chromatography calibration was carried out using a kit from Pharmacia Fine Chemicals Co. Each standard (1.5 ml) was chromatographed with their columns. A Toyo SF-160K rectangular balance-operated fraction collector was used throughout all this work. At this time, two calibration methods were evaluated. First, the highest peak fraction was adopted as elution volume (V_e) (Method I). Second, V_e was determined as the gravity of the elution profile as shown in the following equation:

$$V_e(g) = \Sigma A_i \times V_e(i)/\Sigma A_i \quad (1)$$

where $V_e(g)$, A_i and $V_e(i)$ mean the elution volume calculated by Eqn. 1, the absorbance of i^{th} fraction and the elution volume of the i^{th} fraction, respectively (Method II).

The degradation behavior of DAS-Q50 was estimated by change of molecular weight whose calibration had been obtained from the above procedure. Two samples were prepared. DAS-Q50 (0.752 g) was dissolved in 15 ml of a 0.5% CH_3COONa aqueous solution, pH 7.2 (Sample A) and DAS-Q50 (1.502 g) in 15 ml of a 1/15 M phosphate buffer, pH 7.4 (Sample B).

Change of molecular weight can be estimated precisely by measuring the weight of each fraction content after gel chromatography when heterogeneous degradation, for example, degradation of DAS-Q50 occurs. However, weight measurement procedure takes a long time and is inconvenient practically. So, optical measurement for determination will be desirable. In this work, the application of the phenol sulfuric acid method was attempted and evaluated. First, the elution pattern estimated by the phenol sulfuric acid method was compared with that by the weight measurement method. Then, the following equation was used for fitting of each pattern.

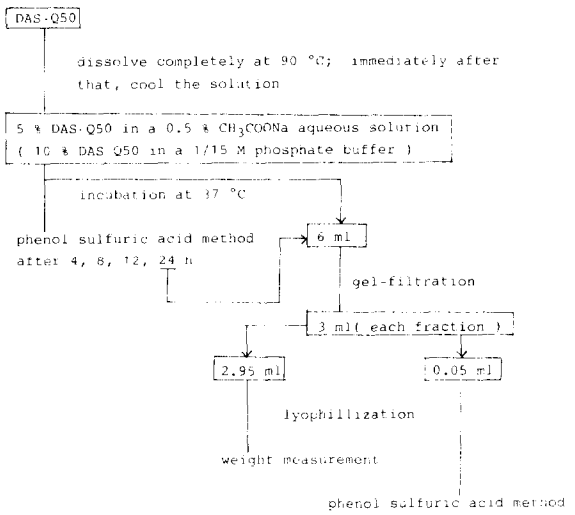
$$\Sigma(A_i(obs) - K_b \times W_i)^2 \quad (2)$$

K_b was a coefficient determined by the least-squares to equation 2, where $A_i(obs)$ and W_i represent absorbance at 490 nm by the phenol sulfuric acid method and weight of i^{th} fraction. $A_i(cal)$ was a calculated value given by $K_b \times W_i$. Then, the discrepancy between the estimation by the phenol sulfuric acid method and that by weight measurement was evaluated by the following equations:

$$\Sigma |A_i(obs) - A_i(cal)| / \Sigma A_i(obs) \quad (3)$$

$$(1/n)\Sigma[\,|A_i(obs) - A_i(cal)|/A_i(obs)] \quad (4)$$

Next, the effect of DAS-Q50 degradation on the phenol sulfuric acid method was investigated. Aliquot samples were withdrawn after 0, 4, 8, 12 and 24 h incubation and the phenol sulfuric acid method was applied. After $E^{1\%}_{1cm}$ values were calculated for each sample, they were compared. All above procedure is shown in Scheme 1.



Scheme 1. Procedure for the estimation of the degradation behavior by weight measurement and by the phenol sulfuric acid method.

Effect of pH and temperature on the dissolution and the degradation of DAS-Q50

DAS-Q50 (50 mg) was added into 30 ml of a 1/15 M phosphate buffer and stirred magnetically at 1800 rpm. These operations were carried out under four conditions: pH 6.5, 20°C; pH 6.5, 40°C; pH 7.4, 20°C; and pH 7.4, 40°C. The dissolution time, which is defined as the time for a sample to dissolve completely, was checked. In addition, the degradation behaviors were investigated by the Sephadex G100 columns whose void volume fraction (V_0) was No. 5 and ($V_0 + V_i$) fraction was No. 13, where V_i meant the gel internal volume. The elution patterns were estimated by the phenol sulfuric acid method immediately after complete dissolution and after a further 24 h incubation.

Confirmation of binding and composition of DAS-Q50–INAH

The content of INAH in DAS-Q50-INAH was estimated by N/C molar ratio after elementary analysis. Moreover, immediately after DAS-Q50–INAH (40.25 mg) was dissolved in 20 ml of a 0.1 N NaOH aqueous solution, the gel chromatography was performed using a Sephadex G50 column where V_0 was No. 19 and ($V_0 + V_i$) was No. 50. The eluted DAS-Q50–INAH was estimated spectrophotometrically at 267 nm and the binding between high molecular DAS-Q50 and INAH was calculated by elementary analysis to several fractions.

Evaluation of in vitro dissolution of DAS-Q50–INAH and the released compounds

DAS-Q50–INAH (1.001 g) was added to 50 ml of a 1/15 M phosphate buffer, pH 7.4 which was prepared with D_2O instead of H_2O. This suspension was stirred constantly at 1000 rpm at 37°C. Each aliquot sample (5 ml) was withdrawn after 3, 8, 13, 18, 23 and 28 days and replaced by the same volume of the dissolution fluid. After 3 ml was taken from each sample and freeze-dried, the total released content of INAH was calculated from the weight and its contained nitrogen ratio.

The property of INAH skelton release was checked by NMR under the similar condition where DAS-Q50–INAH (2.004 g) stirred in the above phosphate buffer, pH 7.4 (100 ml) at 1000 rpm and at 37°C. Aliquot samples (5 ml) were withdrawn at 3, 6, 9, 12, 15 and 33 days and the buffer (5 ml) was supplied after each sampling. D_2O buffer (0.2 ml) containing methanol (0.79 mg) was added as a standard in 1 ml of each sample. The molar ratio of INAH skelton protons to methyl protons of methanol gave the content of INAH skeltons. However, this calculated value was used for comparison with the estimation by weight and nitrogen ratio because it was difficult to identify released compounds completely.

The released compounds were estimated by employing the TLC method following a similar operation in which DAS-Q50–INAH (400 mg) was added to 20 ml of a 1/15 M phosphate buffer, pH 7.4 and stirred at 1000 rpm at 37°C. Aliquot samples were withdrawn at the appropriate time intervals and used for TLC, while the same volume of dissolution fluid was supplied after each sampling. INAH (2 mg/ml), INA (2.1 mg/ml) and

INAA (2.1 mg/ml) were prepared as standards. Ten drops of all samples were placed on a TLC plate with 2/1 (v/v) $C_2H_5OH/CHCl_3$. Concerning intact INAH, a similar experiment was carried out under the same conditions except that INAH (400 mg) was used instead of DAS-Q50–INAH (400 mg). Aliquot samples were checked by TLC. The fluorescence technique and ninhydrin reaction were used for detection.

Results and Discussion

DAS-Q50 particles had an ellipsoid and the surface was smooth. Maximum dimensions ranged from a few micrometers to 80 μm. The mean value was 29.4 μm. X-Ray diffraction data gave an amorphous pattern. These data suggest that DAS-Q50 might dissolve readily.

The calibration between the elution volume (V_e) and the molecular weight (M) in gel chromatography was reported by P. Andrews (1964). Frequently, it is estimated by using log M and K_{av}. K_{av} is given by the equation:

$$K_{av} = (V_e - V_0)/(V_t - V_0) \quad (5)$$

where V_t is the total bed volume. The calibration between $(V_e - V_0)$ and log M is consistent with Eqn. 5. The latter calibration was employed in this study. Method II gave a better correlation coefficient than Method I. These results are shown in Fig. 1. The molecular weight distribution of DAS-Q50 could be estimated approximately under a 0.5% CH_3COONa aqueous solution condition because of the slower degradation. This is given in Fig. 2. The recovery rate obtained by gel filtration was 86% immediately after dissolution and 86% after further 24 h incubation. Immediately after dissolution, 56% of the total collected sample had more than 10^4 molecular weight. It was supposed that most DAS-Q50 had a high molecular weight of more than 10^4. Even after further 24 h incubation, 23% had more than 10^4 molecular weight. Under a 1/15 M phosphate buffer, pH 7.4 condition, 29% of the total collected sample showed more than 10^4 molecular weight immediately after dissolution. After further 24 h incubation, only 2%

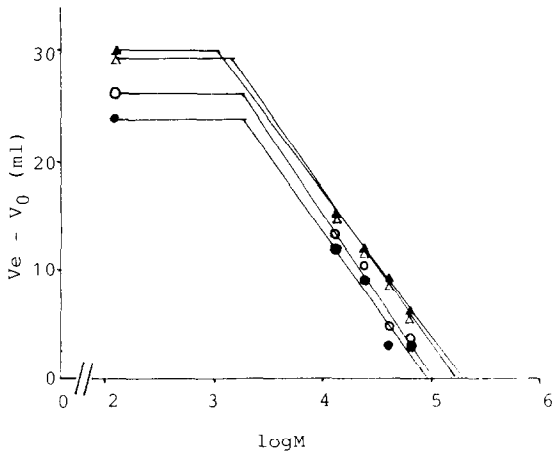


Fig. 1. Linear calibration of molecular weights. Key: (▲) in a 0.5% CH_3COONa aqueous solution, pH 7.2 by Method I and $(V_e - V_0) = 68.82 - 12.964 \cdot \log M$, $r = -0.998$; (△) in a 0.5% CH_3COONa aqueous solution, pH 7.2 by Method II and $(V_e - V_0) = 72.98 - 13.72 \cdot \log M$, $r = -0.999$; (●) in a 1/15 M phosphate buffer, pH 7.4 by Method I and $(V_e - V_0) = 71.90 - 14.483 \cdot \log M$, $r = -0.957$; (○) in a 1/15 M phosphate buffer, pH 7.4 by Method II and $(V_e - V_0) = 75.38 - 14.697 \cdot \log M$, $r = -0.977$.

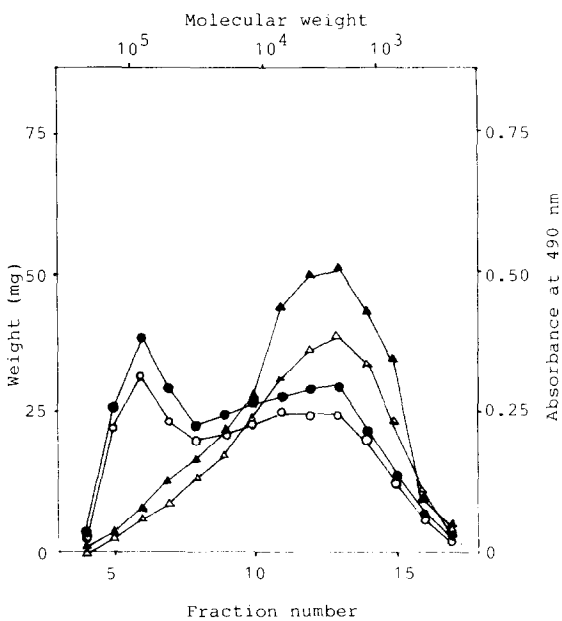


Fig. 2. Elution patterns of DAS-Q50 in a 0.5% CH_3COONa aqueous solution, pH 7.2. Key: (○) immediately after the dissolution and by weight measurement; (△) after further 24 h incubation at 37°C and by weight measurement; (●) immediately after dissolution and by the phenol sulfuric acid method; (▲) after further 24 h incubation at 37°C and by the phenol sulfuric acid method.

had more than 10^4 molecular weight. This fact suggests that DAS-Q50 would degrade quickly non-enzymatically. DAS-Q50 may be degradable under parenteral administration. It will be difficult to utilize DAS-Q50 as a stable and long-action adsorption agent in a body. So, in this study, the utilization to chemical drug trapping will be suggested because of the erodibility and the pharmacuetical properties will be checked.

The elution patterns of DAS-Q50 were estimated by weight measurement and by the phenol sulfuric acid method. The comparison betwen the pattern by the phenol sulfuric acid method and that by the weight measurement method is shown in Table 1. Each pattern agreed very well, the discrepancy index from the Eqns. 3 and 4 being less than 10.0%. Table 2 indicates that the absorbance at 490 nm after the phenol sulfuric acid method was independent of incubation time or degradation. $E^{1\%}_{1cm}$ was 77.23 in a 1/15 M phosphate buffer, pH 7.4, in which the maximum error was 2.4%. $E^{1\%}_{1cm}$ was 79.98 in a 0.5% CH_3COONa aqueous solution, pH 7.2, in which the maximum error was 9.2%. Therefore, the effect of the degradation on the phenol sulfuric acid method was found to be scarcely traceable and that can be adopted to estimate the elution pattern of DAS-Q50 and to pursue the degradation behavior.

DAS-Q50 did not dissolve completely in the deionized water even by stirring for 7 days at 1800 rpm. The dissolution time under several conditions in a 1/15 M phosphate buffer was obtained as shown in Table 3. The degradation behaviors are described in Fig. 4. Sample D whose condition was very close to in vivo, dissolved completely within one hour and degraded the quickest. Dis-

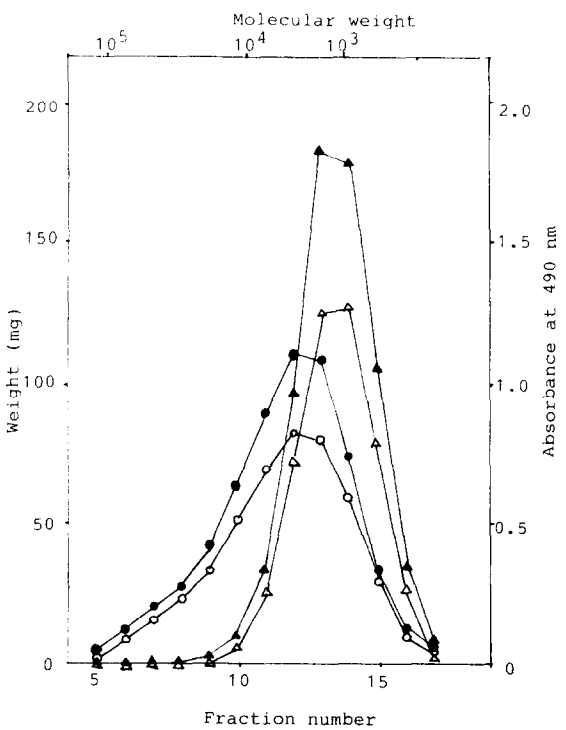


Fig. 3. Elution patterns of DAS-Q50 in a 1/15 M phosphate buffer, pH 7.4. Key: (○) immediately after the dissolution and by weight measurement; (△) after further 24 h incubation at 37°C and by weight measurement; (●) immediately after the dissolution and by the phenol sulfuric acid method; (▲) after further 24 h incubation at 37°C and by the phenol sulfuric acid method.

TABLE 1

COMPARISON BETWEEN THE WEIGHT MEASUREMENT AND THE PHENOL SULFURIC ACID METHOD

	Condition			
	1/15 M phosphate buffer, pH 7.4		0.5% CH_3COONa solution, pH 7.2	
	0 h	24 h	0 h	24 h
$\frac{\Sigma\lvert A_{obs} - A_{cal}\rvert}{\Sigma A_{obs}}$	0.047	0.064	0.041	0.071
$\frac{1}{n}\Sigma\left(\frac{\lvert A_{obs} - A_{cal}\rvert}{A_{obs}}\right)$	0.064	0.074	0.046	0.097

TABLE 2

CHANGE $E^{1\%}_{1cm}$ VALUE ON THE PHENOL SULFURIC ACID METHOD AT EACH TIME

Time (h)	$E^{1\%}_{1cm}$	
	1/15 M phosphate buffer, pH 7.4	0.5% CH_3COONa solution, pH 7.2
0	79.08	84.41
4	75.95	75.45
8	78.07	75.55
12	78.52	87.33
24	75.54	77.16

TABLE 3

DISSOLUTION TIME UNDER THE SEVERAL CONDITIONS

Sample	Condition		Dissolution time (h)
	pH	Temperature (°C)	
A	6.5	20	37.0
B	6.5	40	2.4
C	7.4	20	12.0
D	7.4	40	0.8

solution and degradation were found to be affected by pH and temperature.

It was found that DAS-Q50 degraded along with dissolving. So, if the reaction between DAS-Q50 and a drug takes place in suspension, degradation may be suppressed. In the case of INAH as a drug, high reactivity was detected under the DAS-Q50 suspension condition. The product (7.4 g) was obtained from DAS-Q50 (5.4 g) and INAH (4.6 g). INAH-loaded DAS-Q50 was named DAS-

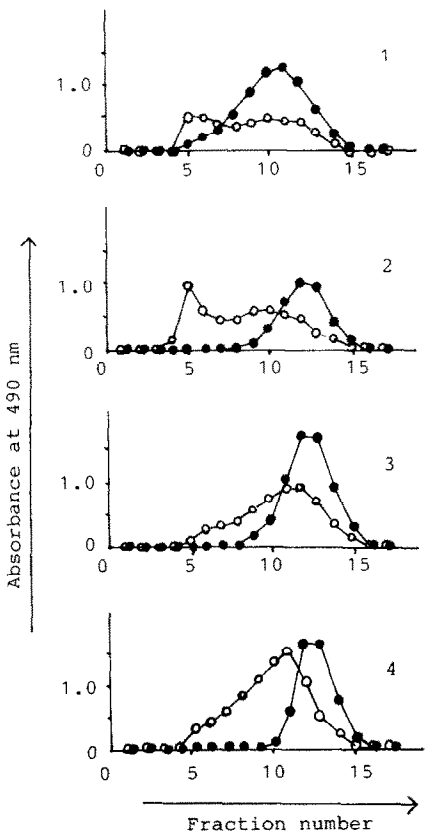


Fig. 4. Elution patterns of DAS-Q50 under various degradation conditions. Key (1) Sample A; (2) Sample B; (3) Sample C; (4) Sample D. (○) immediately after the complete dissolution; (●) after further 24 h incubation at 37°C.

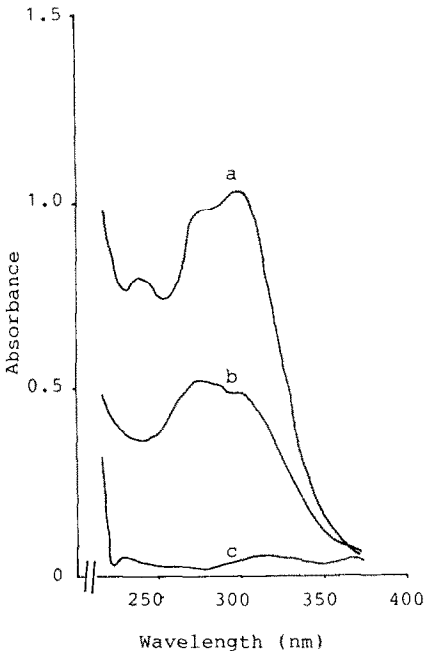


Fig. 5. Ultraviolet spectra profiles of INAH (32 μg/ml) (a); DAS-Q50–INAH (31 μg/ml) (b); DAS-Q50 (31 μg/ml) (c), under a 0.1 N NaOH aqueous solution condition.

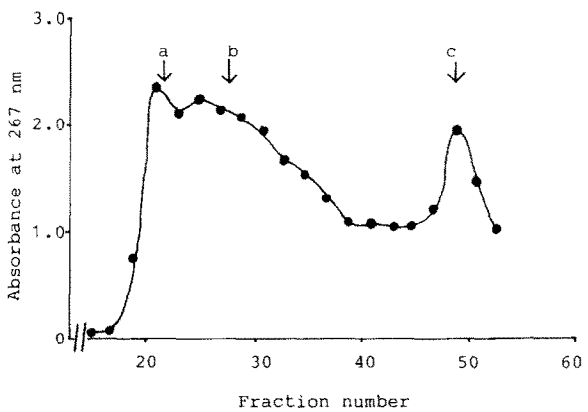


Fig. 6. Confirmation of the binding between DAS-Q50 and INAH. Key: molar ratio (N/C), N/C = 1/10 (a); N/C = 1/5 (b); N/C = 1/5 (c).

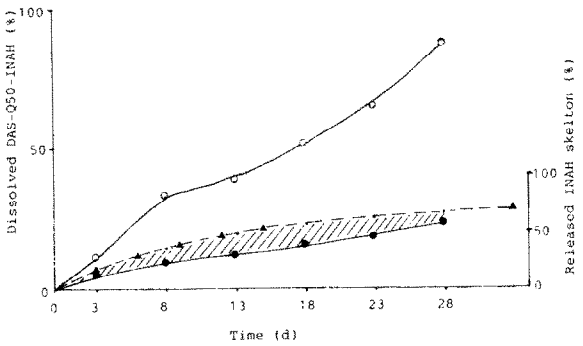
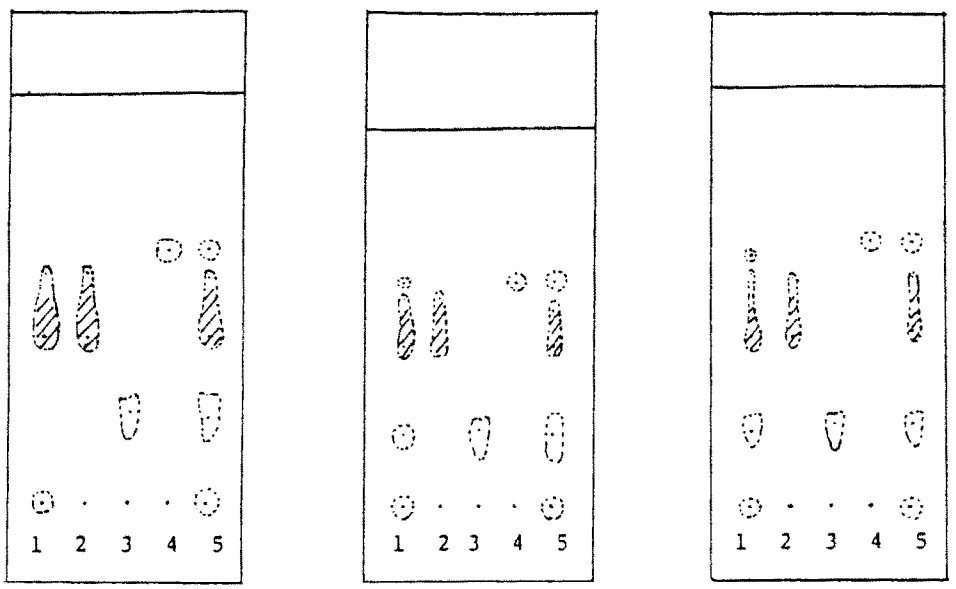


Fig. 7. The dissolution profile of DAS-Q50–INAH and the released INAH skelton. Key: (○) the dissolved DAS-Q50–INAH; (●) the released INAH skelton estimated by weight and nitrogen content; (▲) the released INAH skelton estimated from NMR.

Q50–INAH. The N/C ratio of DAS-Q50–INAH was 2/9 which indicated that 1 mole INAH was bound to 5/4 moles aldehyde group. The content of INAH in DAS-Q50–INAH is shown as in the following equation.

$$W_i = 0.405 \times W_0 \quad (6)$$

where W_i and W_0 mean the content of INAH and the weight of DAS-Q50–INAH, respectively. X-Ray diffraction and electron micrograph showed similar properties to those of intact DAS-Q50. The ultraviolet spectra profiles of DAS-Q50–INAH (31 μg/ml), DAS-Q50 (31 μg/ml) and INAH (32 μg/ml) under a 0.1 N NaOH aqueous



1 day

Rf 1; 0.0, 0.40
2; 0.40
3; 0.21
4; 0.61
5; 0.0, 0.20, 0.40, 0.61

1 ,(A_{263}= 0.41)

4 days

Rf 1; 0.0**, 0.18†, 0.42, 0.58*
2; 0.42
3; 0.20
4; 0.58
5; 0.0, 0.18, 0.42, 0.58

1 ,(A_{263}= 0.68)

10 days

Rf 1; 0.0**, 0.18†, 0.40, 0.60*
2; 0.40
3; 0.19
4; 0.63
5; 0.0, 0.19, 0.42, 0.63

1 ,(A_{263}= 1.20)

1; dissolved compounds, 2; 2.0mg/ml INAH, 3; 2.1mg/ml INA, 4; 2.1mg/ml INAA

○ ; F_{254} + , ⁂ ; ninhydrine + , *; weak, **; strong 5; (1+2+3+4)

* N/C(molar ratio) at Rf(0.0) = 1/9 to 8 days stirred sample, obtained

Fig. 8. Evaluation of the released compounds by TLC.

solution are shown in Fig. 5. DAS-Q50 showed little absorption. The result of the gel filtration of DAS-Q50–INAH in a 0.1 N NaOH aqueous solution is described in Fig. 6. Most of the fractions, except some with small molecules, showed the same spectra profile. These spectra profiles corresponded to DAS-Q50–INAH. Moreover, it was found that high molecular DAS-Q50 was bound to INAH, which was judged from the N/C molar ratio of several fractions.

The dissolution behavior of DAS-Q50–INAH is one of its most important pharmaceutical properties. DAS-Q50–INAH dissolved gradually and almost linearly for one month in vitro as shown in Fig. 7. However, some insoluble compounds remained. From the TLC result, the estimation of the dissolved compounds by applying the ultraviolet absorption method was difficult because several compounds were found to have been released. Therefore, in this study, the dissolved compounds were measured by weight after freeze-drying and the total released INAH skelton was calculated from the nitrogen content. The TLC data is shown in Fig. 8. Even after stirring for 3 days, only 13.2% of the total contained INAH was estimated to have dissolved. The release of DAS-Q50–INAH fragment was detected as an origin point from TLC. After 28 days, the INAH skelton (195.2 mg) which corresponded to 48% of the total contained INAH was estimated to remain in the insoluble compounds. Concerning intact INAH, the TLC data gave a strong position only to INAH after 10 and 15 days. INAH was quite stable under this condition. No spot was found at the origin in this incubation.

The NMR data are estimated as follows: the dissolved compounds showed a similar NMR spectra pattern throughout the dissolution test. The ratio of the integrated intensity around 8.6 ppm to that around 7.7 ppm was almost 1 : 1 and the broadened signals derived mainly from DAS-Q50 appeared at 3.4–4.4 ppm. Probably, the low field signal represented mainly the protons of the INAH ring. Methyl protons of methanol used as

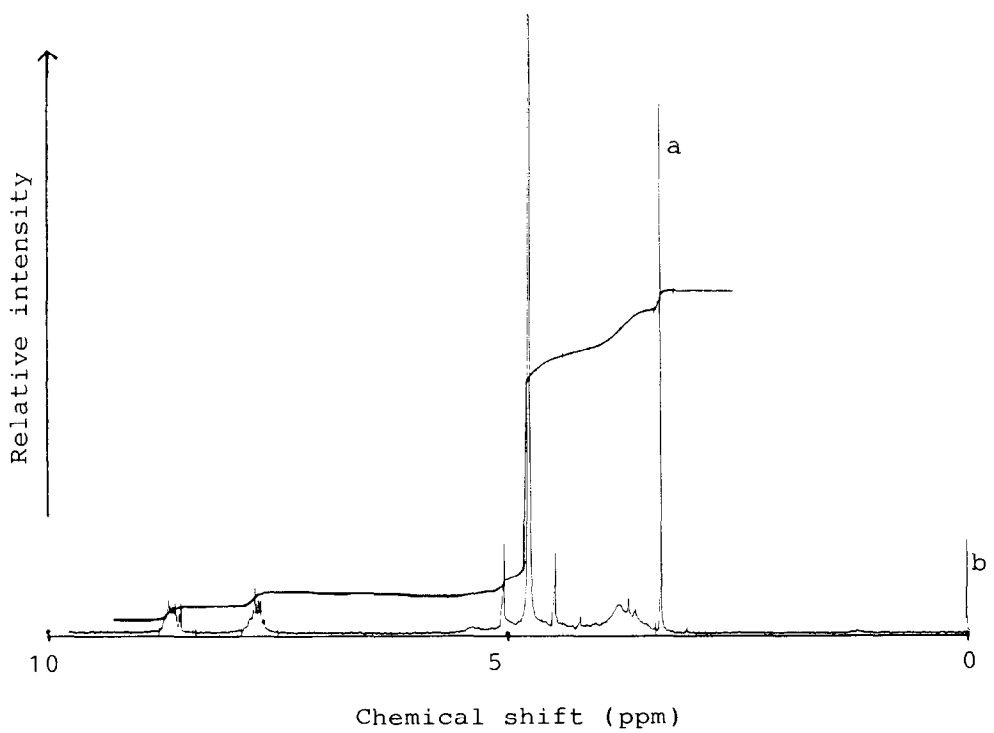


Fig. 9. NMR spectra of the dissolved compounds of DAS-Q50–INAH after the incubation for 15 days. Key: (a) methyl protons of methanol added as an internal standard; and (b) tetramethylsilane (reference).

an interval standard appeared at 3.3 ppm and could be identified easily. Since the NMR spectra profile of 3 days released compounds was very similar to that of free INAH, free INAH was supposed to be released mainly, being consistent with the TLC result. As the further incubation gave the broadened signals at 8.4–8.8 ppm and at 7.5–7.9 ppm almost equivalently in intensity, INAH skelton was supposed to be released being in different forms from free INAH. At the same time, signals derived from DAS-Q50 came to appear. These observations were consistent with TLC data. If the low field signals (8.4–8.8 ppm and 7.5–7.9 ppm) show only the protons of the INAH ring, the released INAH skelton could be calculated precisely from the ratio of the low field signal intensity to that of methyl protons of methanol. However, since the released compounds were complicated and the signal assignment was not identified completely, the calculated values of released INAH skelton by the NMR method was adopted as a reference to the estimation by weight and nitrogen content. The estimated values by the NMR method has a tendency to overestimate but never to underestimate because the low field signals contain the protons of INAH skelton at least. The values to 8, 13, 18, 23 and 28 days were calculated by the linear interpolation of the native NMR data. These showed a larger value by 5–15% of contained INAH than the estimations by weight and nitrogen content. From the NMR data, the burst release phenomena of INAH skeleton were not observed. This was consistent with the TLC data which indicated that INAH decomposition was quite small. Anyway, INAH skelton was found to be released gradually for about one month along with DAS-Q50–INAH dissolution. As the method by weight and nitrogen content does not overestimate the released INAH skelton at all, the true release of INAH skelton exists in the shade line zone in Fig. 7. The dissolved compounds were recognized to be small molecules by gel chromatography.

Based on this discussion, DAS-Q50–INAH has a property to act as a sustained release drug delivery system with the gradual dissolution of the conjugate. A sustained release system would be expected generally in compounds between DAS and drugs having a hydrazino-group.

Acknowledgements

We are very grateful to the Japan Carlit Co. for supplying DAS. We thank Mr. Yoshiaki Machida and Miss Junko Okuda for their assistance in the experimental work.

References

Andrews, P., Estimation of the molecular weights of proteins by Sephadex gel-filtration. Biochem. J., 91 (1964) 222–233.

Barry, V.C. and Mitchell, P.W.D., Properties of periodate-oxidised polysaccharides. Part I. The preparation of polymeric substances containing nitrogen from oxidised starches. J. Chem. Soc., (1953) 3610–3612.

Barry, V.C. and Mitchell, P.W.D., Properties of periodate-oxidised polysaccharides. Part IV. The products obtained on reaction with phenylhydrazine. J. Chem. Soc., (1954) 4020–4023.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F., Colorimetric method for determination of sugars and related substances. Anal. Chem., 28 (1956) 350–356.

Mester, L., The formazan reaction in proving the structure of periodate oxidized polysaccharides. J. Am. Chem. Soc., 77 (1955) 5452–5453.

Shimizu, T., The urea adsorption agent. Kokai Tokkyo Koho, Sho57-130545 (JP, A) (1982).

Shimizu, T., Fujishige, S. and Okada, A., Particles of activated oxidized polysaccharide substances coated with inactive protective layer and method for manufacture thereof. Tokkyo Koho, Sh57-29447 (JP, B2) (1982a).

Shimizu, T., Fujishige, S. and Okada, A., Particles activated oxidized polysaccharide substance coated with inactive protective layer and method for manufacture thereof. U.S. Patent 4339360 (1982b).

Watanobe, T. and Shibuya, M., Determination of carbonyl groups in dialdehyde starch by sodium borohydride method. Japan Analyst, 15 (1966) 176–179.

Whistler, R.L., Chang, P.K. and Richards, G.N., Alkaline degradation of periodate-oxidized starch. J. Am. Chem. Soc., 81 (1959) 3133–3136.