CHAPTER IV

RESULTS AND DISCUSSION

1. Synthesis and characterization of succinylated zidovudine

The preparation of dextrin-zidovudine conjugate involved two synthetic steps. Firstly, zidovudine was succinylated to introduce a carboxylic functional group by reacting with succinic anhydride in the presence of triethylamine. Zidovudine was characterized by FT-IR and ^{1}H-NMR as shown in figures 56 and 60, appendix I, respectively.

1.1 Synthesis of succinylated zidovudine

Succinic anhydride modification has been widely used as the mean of introducing carboxylic groups. In this method, zidovudine was reacted with succinic anhydride according to the Giammona's method (Giammona et al., 1998). The mole ratio of succinic anhydride to zidovudine was adjusted from 3 18: 1 to 2.10: 1. The amount of succinic anhydride was reduced due to an excess adding of succinic anhydride leads to difficulty in removing unreacted succinic anhydride, which appeared as an impurity in the product on completion of the reaction. The succinic anhydride was detected as yellow color on TLC plate staining with bromocresol green solution.

For purification, free zidovudine was removed by extraction with ethyl acetate. Solvent extraction technique could not remove all unreacted zidovudine, so the product was further purified by flash column chromatography packed with sephadex LH-20. Flash column chromatography technique using sephadex LH-20 as the stationary phase can separate compounds in the mixture according to the molecular weight of the individual molecule. Succinic anhydride has molecular weight of 100 and zidovudine has molecular weight of 267.2 whereas succinylated

zidovudine has molecular weight of 367.2. Therefore, sephadex LH-20 can separate these compounds out of the mixture. It was found that sephadex LH-20 provided better separation for this mixture than silica gel. The mobile phase for eluting the column was the mixture of chloroform and methanol in the ratio of 9:1, v/v. Fractions collected from the column were detected by TLC technique. The TLC plate was developed by solvent mixture of chloroform and methanol in the ratio of 9:1, v/v. There were two spots on TLC plate when detected under UV light at 254 nm that were zidovudine and succinylated zidovudine. Zidovudine has Rf value of 0.30 and succinylated zidovudine has Rf value of 0.16. When the TLC plate was stained with bromocresol green and detected under UV light at 254 nm, the succinic impurity was detected as yellow color. The fractions that contained only succinylated zidovudine were combined and solvent was removed by evaporation. The purification by sephadex LH-20 flash column chromatography could remove free zidovudine and free succinic anhydride and provided high purity (> 97%) of succinylated zidovudine as shown at retention time of 25.782 min in figure 15. Only 2 % of free zidovudine remained as shown at retention time of 20.024 min in figure 15. The percentage yield of succinylated zidovudine was obtained approximately 60 %. Succinylated zidovudine was characterized by FT-IR and ^{1}H-NMR as shown in figures 57 and 61, appendix I and also by elemental analysis.

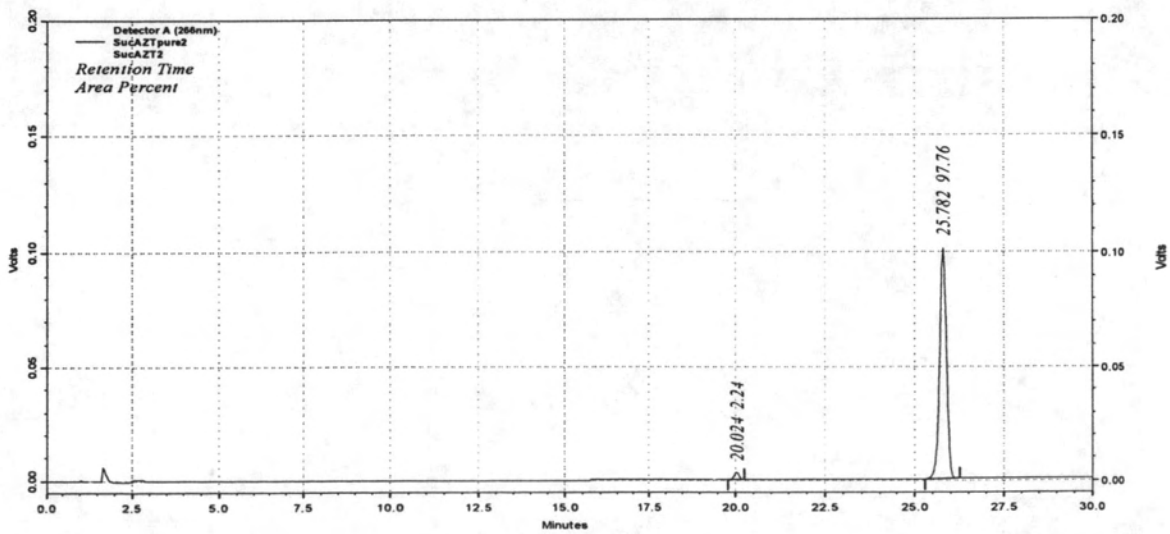


Figure 15 HPLC chromatogram indicating high purity of resulting succinylated zidovudine.

1.2 Characterization of succinylated zidovudine

1.2.1 Infrared spectroscopy (IR)

The FT-IR spectrum of succinylated zidovudine is shown in figure 57, appendix I. The FT-IR spectrum exhibited a broad band of O-H stretching in the range of 3660 – 2700 cm^{-1} centered at 3190 cm^{-1}. The azido group ($-N_3$) appeared at 2110 cm^{-1}. The carbonyl stretching of ester of carboxylic acid showed at 1735 cm^{-1}. The C=O band was a broad band due to the overlap of two esters of the carboxylic acid and of 6 pie-position owing to the incorporation of succinic groups together with carbonyls of -CO-NH-CO- of zidovudine.

1.2.2 Nuclear magnetic resonance spectroscopy (NMR)

The ^{1}H-NMR in $CDCl_3$ is shown in figure 61, Appendix I. The spectrum showed the presence of hydrogens of succinic group at δ = 2.62 and hydrogen of carboxylic group at δ = 10.62. The FT-IR and ^{1}H-NMR spectra showed the successful incorporation of the succinic group to zidovudine.

1.2.3 Elemental analysis

Calculated percentages of C, N, H contents of succinylated zidovudine from chemical formula; $C_{14}H_{17}N_5O_7$ were 45.78 for C, 19.07 for N and 4.67 for H. The resulting succinylated zidovudine was analyzed by elemental analyzer. The observed percentages of C, N, H contents were 45.78, 19.00 and 4.66 respectively. The result from elemental analysis indicated high purity of succinylated zidovudine.

2. Synthesis and characterization of dextrin-zidovudine conjugate

The second step for preparation of dextrin-zidovudine conjugate was using dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt) for coupling dextrin with succinylated zidovudine. Dextrin was characterized by FT-IR and ^{1}H-NMR before reaction as shown in figures 58, 62 and 63, appendix I.

2.1 Synthesis of dextrin-zidovudine conjugate

Succinylated zidovudine bears carboxylic group that is able to covalently link to hydroxyl groups of dextrin. The ester bond was formed between succinylated zidovudine and dextrin. Dextrin contains three hydroxyl groups per one unit that are available for chemical modification. The most reactive hydroxyl group is at C6 position due to less steric hindrance; therefore, the reaction occurs at this position more than other hydroxyl groups. The resulted product was dextrin-zidovudine conjugate in which dextrin backbone and zidovudine were linked by succinic spacer via two ester linkages as shown in figure 16.

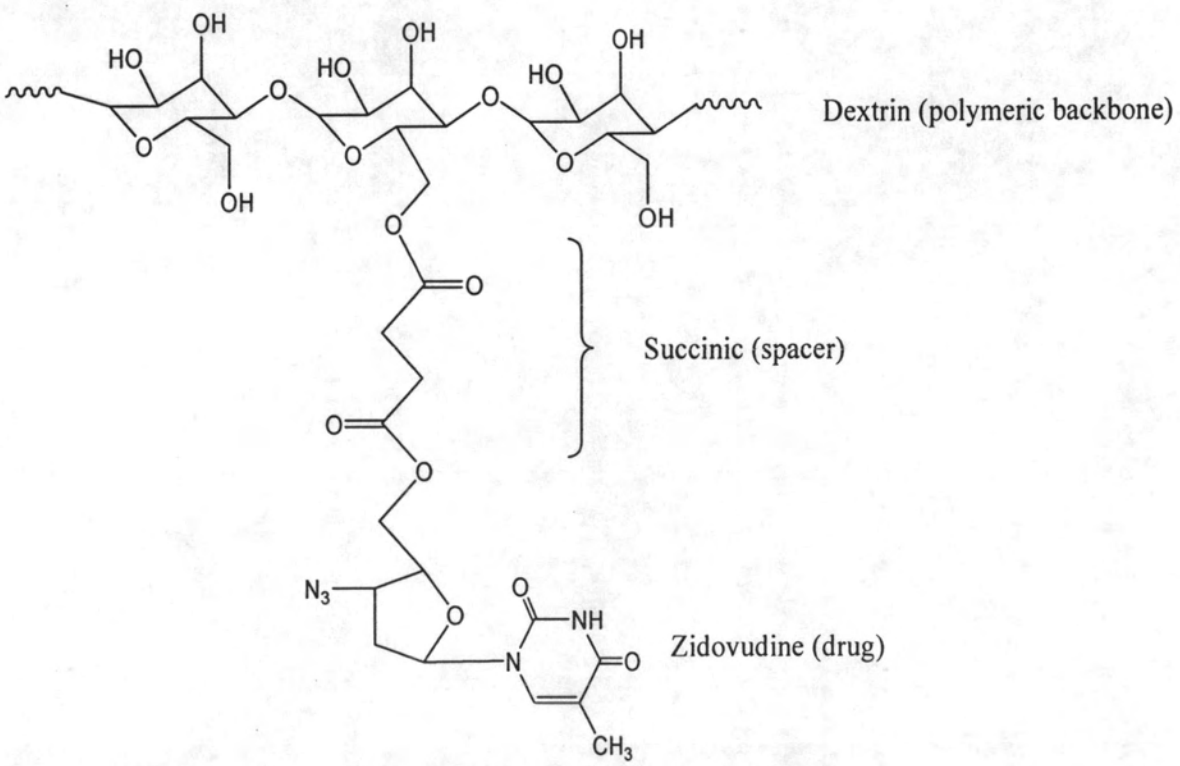


Figure 16 Dextrin-zidovudine conjugate containing succinic spacer.

The conjugation of succinylated zidovudine and dextrin was carried out in the presence of DCC and HOBt as coupling agents (Ahn et al., 1998, Pakalns et al., 1999, Masuko et al., 2005) and *N, N*-dimethylaminopyridine (DMAP) was used as a catalyst (Hreczuk-Hirst, German, Duncan, 2001). The molar ratio of dextrin: succinylated zidovudine in the reaction was 2.5:1. The solution of dextrin was prepared by dissolved in DMF with small amount of DMSO. The volume of DMSO was small due to it was difficult to remove after reaction but it is necessary for dissolving dextrin. The progression of reaction was followed by TLC. Dextrin-zidovudine conjugate had Rf value of 0. The suitable reaction time was 48 h at room temperature. After the product was purified by dialysis and dried by freeze-drying technique, the percentage yield of product was approximate 87 %. The product was characterized by FT-IR and ^{1}H-NMR spectroscopy as shown in figures 59 and 64 in appendix I, respectively.

2.2 Characterization of dextrin-zidovudine conjugate

2.2.1 Infrared spectroscopy (IR)

The structure of the dextrin-zidovudine conjugate was confirmed by FT-IR and ^{1}H-NMR. The FT-IR spectrum (figure 59, appendix I) showed the absorption of the $-N_3$ group of succinylated zidovudine at 2110 cm^{-1}. The ester of succinic spacer absorbed at 1734 cm^{-1} whereas the amide I and amide II absorptions of succinylated zidovudine occurred at 1647 cm^{-1} and 1568 cm^{-1}, respectively. The remaining unreacted hydroxyl groups of dextrin appeared as a broad band centered at 3401 cm^{-1}. The CH, CH_2 stretching of dextrin exhibited at 2929 cm^{-1} and the absorption of O-CH_2 of dextrin was shown at 1025 cm^{-1}.

2.2.2 Nuclear magnetic resonance spectroscopy (NMR)

The ^{1}H-NMR in D_2O of the dextrin-zidovudine conjugate (figure 64, appendix II) showed the peak at δ = 1.66 due to methyl protons of zidovudine. The signals between δ = 2.31 and δ = 2.53 were from protons of C2′ of sugar ring of

zidovudine and that of succinic spacer. The peak at δ = 5.94 corresponded to the hydrogen of C1′ of zidovudine and the peak at δ = 7.28 was due to hydrogen of C6 on the pyrimidine ring of zidovudine. The broad bands from δ = 2.94 to δ = 5.45 were contributed to protons of the dextrin polymer. The absence of free zidovudine or succinylated zidovudine in the dextrin-zidovudine conjugate was confirmed by HPLC analysis.

2.2.3 Determination of drug loading on dextrin-zidovudine conjugate

The drug loading into the polymeric backbone was estimated by UV and ^{1}H-NMR spectroscopy. The calculation of drug loading from UV spectroscopy was performed by calculating from calibration curve of zidovudine. The UV spectrum of zidovudine was shown in figure 54, appendix I and calibration curve is shown in figure 17. The UV spectrum of dextrin-zidovudine conjugate is shown in figure 55, appendix I.

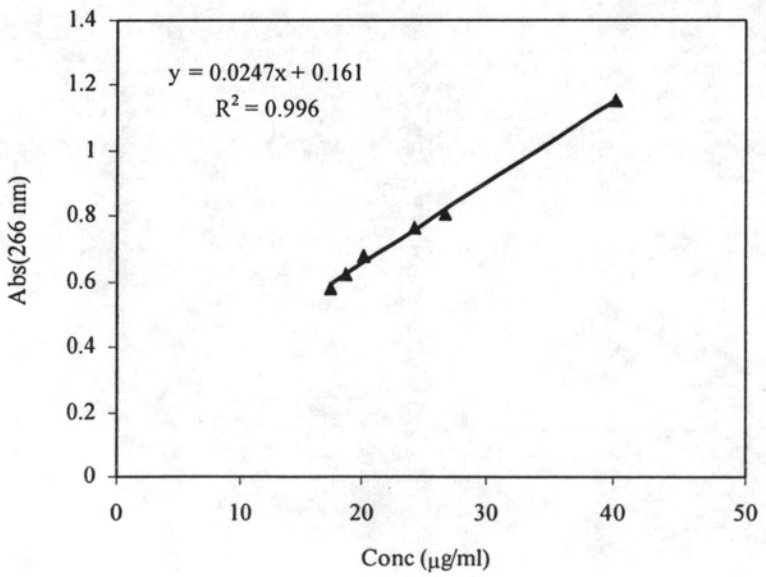


Figure 17 Standard curve of zidovudine in water (UV spectroscopy, λ = 266 nm).

Table 6 UV absorbance of dextrin-zidovudine conjugate.

Concentration of the conjugate (μg/ml)	Absorbance (266 nm) *	Concentration of zidovudine (μg/ml)	Zidovudine loading (% w)
154	0.881	29.15	18.92

* Absorbance value from average of three measured values

The linked zidovudine was 18.92 % w (15.24 % mole). The average linked zidovudine was also confirmed by ^{1}H-NMR. The integrated signals of anomeric protons (proton that near 2 oxygen atoms) of dextrin at $\delta = 4.60$ to $\delta = 5.45$ (as internal reference signal) were compared with the signals of protons of zidovudine at $\delta = 1.66$ (CH_3-C5), $\delta = 5.94$ (H-C1′) and $\delta = 7.28$ (H-C6).

The calculation from ^{1}H-NMR spectrum was followed:

$$\% \text{ mole zidovudine loading} = \frac{x}{5y} \times 100$$

Where x is the sum of the integrated areas of protons of zidovudine at $\delta = 1.66$ (CH_3-C5), $\delta = 5.94$ (H-C1′) and $\delta = 7.28$ (H-C6) and y is the integrated area of anomeric protons of dextrin at $\delta = 4.60$ to $\delta = 5.45$ (figure 64 and table 7).

Table 7 Integrated area of protons of dextrin-zidovudine conjugate for calculation of drug loading.

Integrated area of protons at	Value
$\delta = 1.66$	3.306
$\delta = 5.94$	0.942
$\delta = 7.28$	1.000
$\delta = 4.60$ to $\delta = 5.45$	6.983 (1.664 + 5.319)

Therefore, x = 5.248

y = 6.983

The amount of drug loading determined from ^{1}H-NMR was 15.03 mole % being in good agreement with that from UV analysis.

3. HPLC analysis

The typical HPLC chromatogram of stavudine internal standard, zidovudine and succinylated zidovudine is shown in figure 18.

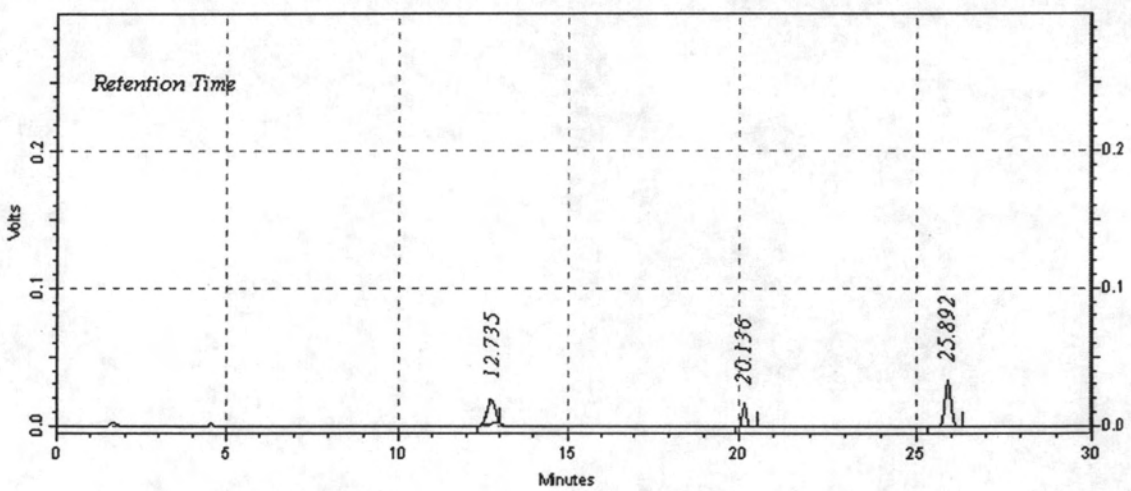


Figure 18 HPLC chromatogram of stavudine internal standard, zidovudine, and succinylated zidovudine

Retention time of Stavudine	=	12.735	min
Retention time of Zidovudine	=	20.136	min
Retention time of Succinylated zidovudine	=	25.892	min

To obtain standard curve of zidovudine, various concentrations of zidovudine containing stavudine internal standard were analysed by HPLC. The area under the peak of zidovudine and that of stavudine were used to obtain peak area ratio of zidovudine/stavudine. The standard curve was constructed by plotting peak area ratio of zidovudine/stavudine internal standard (IS) against various concentrations of zidovudine as shown in figure 19. The standard curve of succinylated zidovudine was constructed as same as zidovudine and is shown in figure 20. These standard curves were used to calculate the concentration of unknown samples.

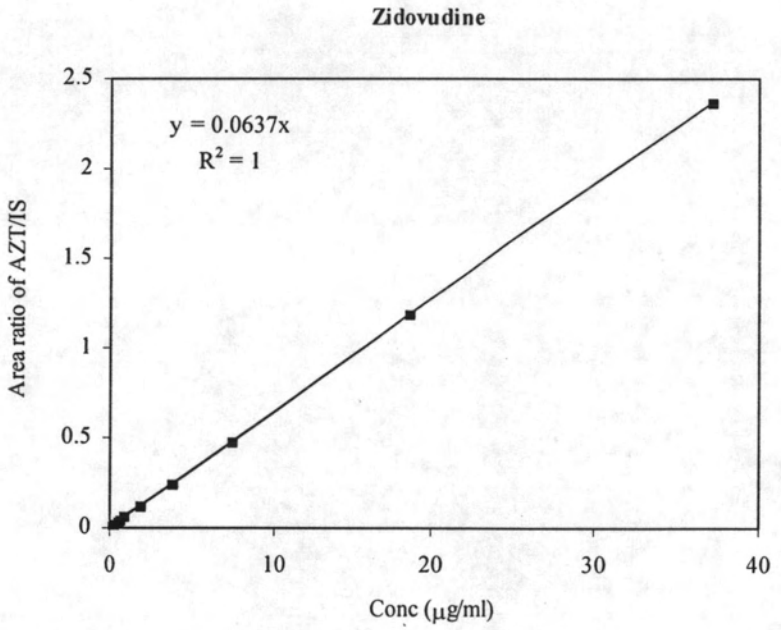


Figure 19 Standard curve of zidovudine

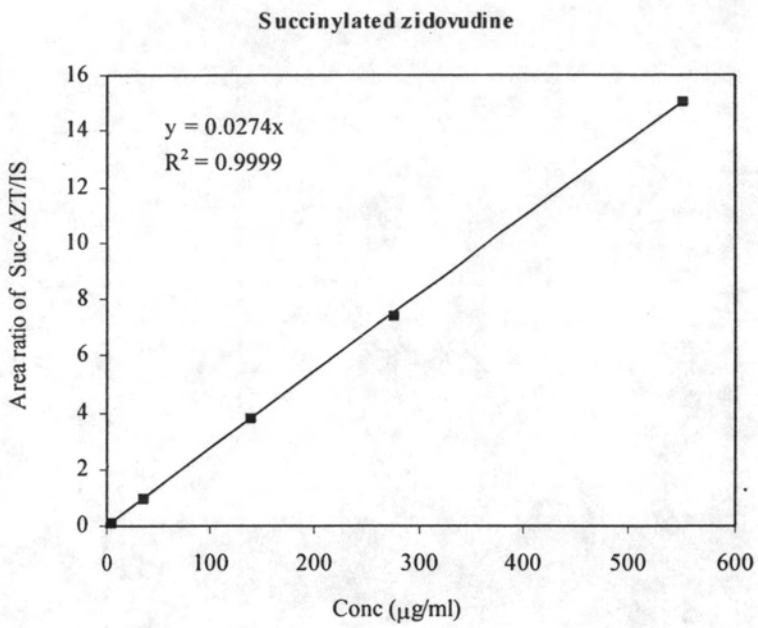


Figure 20 Standard curve of succinylated zidovudine

3.1 Accuracy

The determination of accuracy for analytical method of zidovudine and succinylated zidovudine at three concentrations was processed. The results are shown in tables 8 and 9, respectively.

From table 8, the % recoveries of individual concentration of zidovudine were 105.75, 103.94 and 96.80 % and the % coefficient of variations (% CV) of individual concentration were 0.27, 0.21 and 2.91 %, respectively.

From table 9, the % recoveries of individual concentration of succinylated zidovudine were 105.93, 108.90 and 105.71 % and the % coefficient of variations (% CV) of individual concentration were 1.95, 0.13 and 1.35 %, respectively.

3.2 Precision

3.2.1 Intraday precision

The determination of precision for analytical method of zidovudine and succinylated zidovudine was processed within one day by analysis of six replicates of three concentrations. The results are shown in tables 10 and 11, respectively.

From table 10, the % recoveries of zidovudine at individual concentration were 105.81, 104.26 and 96.68 % and % CV of individual concentration were 0.20, 0.38 and 2.06 %, respectively.

From table 11, the % recoveries of succinylated zidovudine at individual concentration were 107.17, 109.40 and 106.04 % and % CV of individual concentration were 1.94, 0.64 and 0.92 %, respectively.

3.2.2 Interday precision

The determination of precision for analytical method of zidovudine and succinylated zidovudine was processed in different three days by analysis of three replicates of three concentrations. The results were shown in table 12 and 13, respectively.

From table 12, the % recoveries of zidovudine at individual concentration were 109.09, 105.61 and 96.95 % and % CV of individual concentration were 3.44, 1.52 and 2.02 %, respectively.

From table 13, the % recoveries of succinylated zidovudine at individual concentration were 107.32, 109.67 and 108.08 % and % CV of individual concentration were 1.43, 0.81 and 1.97 %, respectively.

Table 8 Accuracy of zidovudine.

Samples	Actual Conc. (μg/mL)	Observed Conc. (μg/mL)	% Recovery	Average % recovery	% CV
A	0.74	0.79	106.09	105.75	0.27
B	0.74	0.78	105.59		
C	0.74	0.78	105.58		
A	5.31	5.51	103.70	103.94	0.21
B	5.31	5.52	104.03		
C	5.31	5.53	104.11		
A	27.88	26.49	95.04	96.80	2.91
B	27.88	27.89	100.05		
C	27.88	26.57	95.31		

Table 9 Accuracy of succinylated zidovudine.

Samples	Actual Conc. (μg/mL)	Observed Conc. (μg/mL)	% Recovery	Average % recovery	% CV
A	1.07	1.15	106.90	105.93	1.95
B	1.07	1.15	107.20		
C	1.07	1.11	103.69		
A	17.67	18.66	108.74	108.90	0.13
B	17.67	18.70	108.95		
C	17.67	18.71	109.00		
A	68.65	71.46	104.08	105.71	1.35
B	68.65	73.00	106.33		
C	68.65	73.27	106.72		

Table 10 Intraday precision of zidovudine.

Samples	Actual Conc. (μg/mL)	Observed Conc. (μg/mL)	% Recovery	Average % recovery	% CV
A	0.74	0.79	105.71	105.81	0.20
B	0.74	0.79	106.09		
C	0.74	0.78	105.59		
D	0.74	0.78	105.58		
E	0.74	0.79	105.91		
F	0.74	0.79	106.01		
A	5.31	5.51	103.70	104.26	0.38
B	5.31	5.52	104.03		
C	5.31	5.53	104.11		
D	5.31	5.54	104.40		
E	5.31	5.55	104.55		
F	5.31	5.57	104.81		
A	27.88	26.49	95.04	96.68	2.06
B	27.88	27.89	100.05		
C	27.88	26.57	95.31		
D	27.88	26.53	95.17		
E	27.88	26.93	96.61		
F	27.88	27.30	97.94		

Table 11 Intraday precision of succinylated zidovudine.

Samples	Actual Conc. (μg/mL)	Observed Conc. (μg/mL)	% Recovery	Average % recovery	% CV
A	1.07	1.15	106.90	107.17	1.94
B	1.07	1.15	107.20		
C	1.07	1.11	103.69		
D	1.07	1.14	106.68		
E	1.07	1.17	108.91		
F	1.07	1.18	109.64		
A	17.67	18.66	108.74	109.40	0.64
B	17.67	18.70	108.95		
C	17.67	18.71	109.00		
D	17.67	18.78	109.41		
E	17.67	18.82	109.64		
F	17.67	18.89	110.65		
A	68.65	71.46	104.08	106.04	0.92
B	68.65	73.00	106.33		
C	68.65	73.27	106.72		
D	68.65	73.15	106.56		
E	68.65	73.00	106.33		
F	68.65	72.94	106.24		

Table 12 Interday precision of zidovudine.

Day	Samples	Actual Conc. (µg/mL)	Observed Conc. (µg/mL)	% Recovery	Average % recovery	% CV
Day 1	A	0.74	0.79	105.71	109.90	3.44
	B	0.74	0.79	106.09		
	C	0.74	0.78	105.59		
Day 2	D	0.74	0.81	108.89		
	E	0.74	0.81	109.54		
	F	0.74	0.83	112.06		
Day 3	G	0.74	0.86	115.71		
	H	0.74	0.82	110.81		
	I	0.74	0.85	114.75		
Day 1	A	5.31	5.51	103.70	105.61	1.52
	B	5.31	5.52	104.03		
	C	5.31	5.53	104.11		
Day 2	D	5.31	5.68	106.93		
	E	5.31	5.58	105.14		
	F	5.31	5.63	105.94		
Day 3	G	5.31	5.76	108.45		
	H	5.31	5.59	105.20		
	I	5.31	5.68	106.95		
Day1	A	27.88	26.49	95.04	96.95	2.02
	B	27.88	27.89	100.05		
	C	27.88	26.57	95.31		
Day 2	D	27.88	26.68	95.70		
	E	27.88	26.82	96.23		
	F	27.88	27.75	99.53		
Day 3	G	27.88	26.65	95.60		
	H	27.88	26.81	96.19		
	I	27.88	27.56	98.86		

Table 13 Interday precision of succinylated zidovudine.

Day	Samples	Actual Conc. (μg/mL)	Observed Conc. (μg/mL)	% Recovery	Average % recovery	% CV
Day 1	A	1.07	1.15	106.90	107.32	1.43
	B	1.07	1.15	107.20		
	C	1.07	1.11	103.69		
Day 2	D	1.07	1.16	107.83		
	E	1.07	1.15	106.91		
	F	1.07	1.16	108.02		
Day 3	G	1.07	1.17	108.75		
	H	1.07	1.16	107.82		
	I	1.07	1.17	108.79		
Day 1	A	17.67	18.66	108.74	109.67	0.81
	B	17.67	18.70	108.95		
	C	17.67	18.71	109.00		
Day 2	D	17.67	19.07	111.09		
	E	17.67	19.04	110.93		
	F	17.67	18.92	110.23		
Day 3	G	17.67	18.73	109.12		
	H	17.67	18.85	109.81		
	I	17.67	18.73	109.14		
Day1	A	68.65	71.46	104.08	108.08	1.97
	B	68.65	73.00	106.33		
	C	68.65	73.27	106.72		
Day 2	D	68.65	74.62	108.69		
	E	68.65	74.47	108.47		
	F	68.65	73.87	107.60		
Day 3	G	68.65	75.57	110.08		
	H	68.65	75.76	110.35		
	I	68.65	75.80	110.41		

3.3 Linearity

The determination of linearity for analytical method of zidovudine and succinylated zidovudine was processed at six concentrations. The results are shown in tables 14 and 15 and figures 21 and 22, respectively.

From table 14 and figure 21, the linearity study of zidovudine showed linear equation of $y = 0.9913x + 0.1208$ with r^2 of 0.9998.

From table 15 and figure 22, the linearity study of succinylated zidovudine showed linear equation of $y = 1.0062x + 2.012$ with r^2 of 0.9999.

3.4 LOQ and LOD

The limit of quantification and the limit of detection for analytical method of zidovudine and succinylated zidovudine were determined. The results of limit of quantification are shown in tables 16 and 17, respectively.

From table 16, the limit of quantification of zidovudine was 0.039 μg/mL and % CV was 9.37 %.

Limit of detection of zidovudine is 0.013 μg/mL that was the concentration that provided signal-to-noise ratio of approximately 3:1.

From table 17, the limit of quantification of succinylated zidovudine was 0.107 μg/mL and % CV was 6.98 %.

Limit of detection of succinylated zidovudine is 0.027 μg/mL that was the concentration that provided signal-to-noise ratio of approximately 3:1.

Table 14 Linearity of zidovudine.

Samples	Actual Conc. (μg/mL)	Observed Conc. (μg/mL)	Average Observed Conc. (μg/mL)
A	0.74	0.79	0.79
B	0.74	0.78	
C	0.74	0.78	
A	5.31	5.51	5.52
B	5.31	5.52	
C	5.31	5.53	
A	7.43	7.54	7.48
B	7.43	7.47	
C	7.43	7.41	
A	18.58	18.60	18.67
B	18.58	18.79	
C	18.58	18.61	
A	27.88	26.93	27.37
B	27.88	27.89	
C	27.88	27.30	
A	37.17	37.12	37.18
B	37.17	37.08	
C	37.17	37.32	

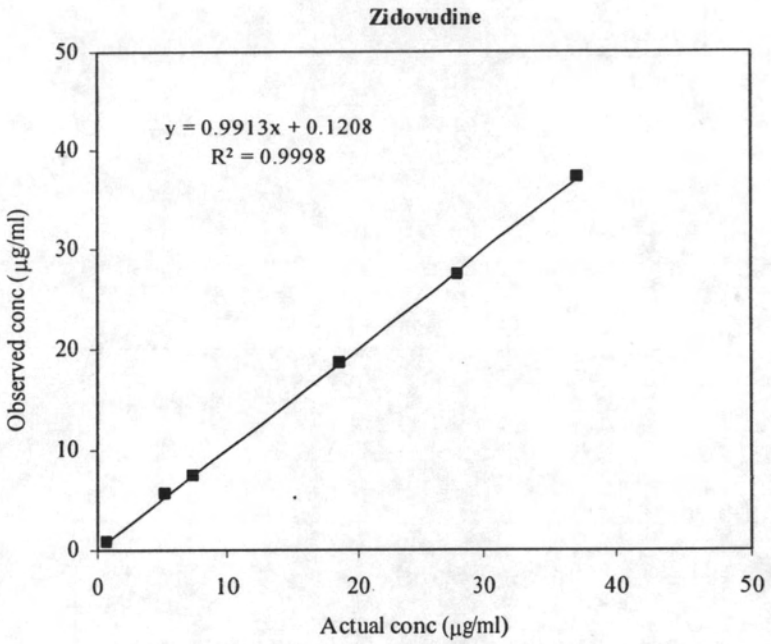


Figure 21 Linearity of zidovudine.

Table 15 Linearity of succinylated zidovudine.

Samples	Actual Conc. (μg/mL)	Observed Conc. (μg/mL)	Average Observed Conc. (μg/mL)
A	1.07	1.11	1.13
B	1.07	1.14	
C	1.07	1.15	
A	17.67	18.66	18.69
B	17.67	18.70	
C	17.67	18.71	
A	34.33	35.44	35.47
B	34.33	35.46	
C	34.33	35.50	
A	68.65	71.46	72.46
B	68.65	73.00	
C	68.65	72.94	
A	274.62	282.99	283.03
B	274.62	282.95	
C	274.62	283.16	
A	549.23	552.19	552.24
B	549.23	552.88	
C	549.23	551.65	

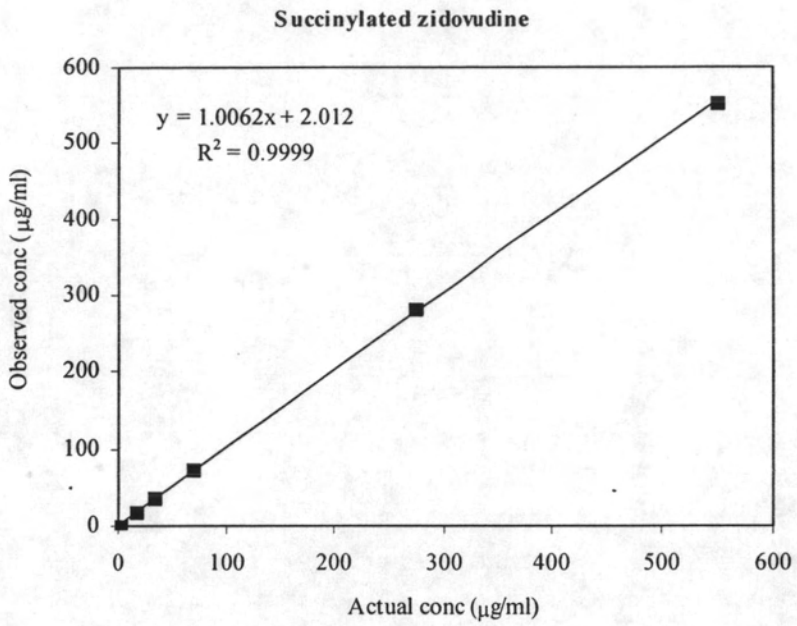


Figure 22 Linearity of succinylated zidovudine.

Table 16 Limit of quantitation of zidovudine.

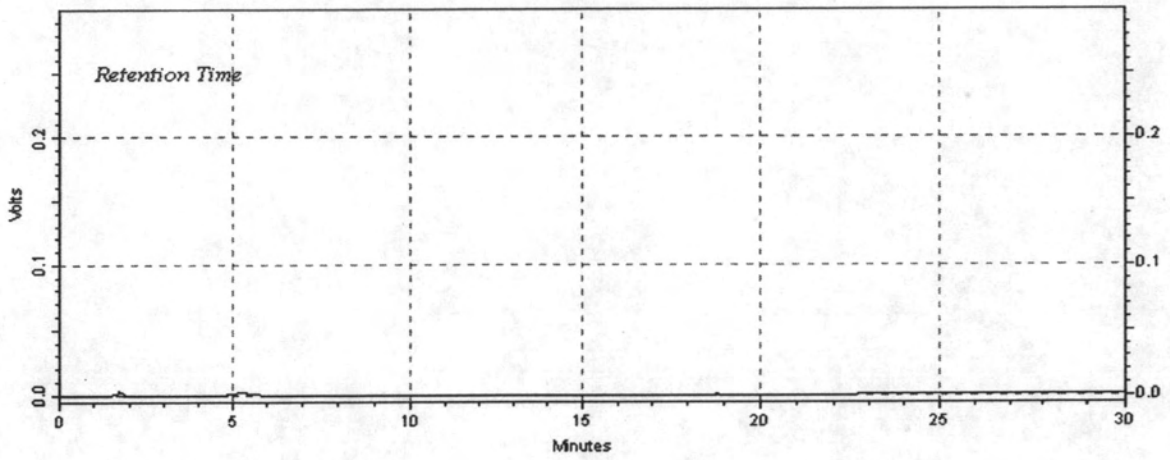
Samples	Actual Conc. (μg/mL)	Observed Conc. (μg/mL)	% Recovery	Average % recovery	% CV
A	0.039	0.040	102.73	93.17	9.37
B	0.039	0.040	102.32		
C	0.039	0.036	93.81		
D	0.039	0.031	79.88		
E	0.039	0.036	92.37		
F	0.039	0.034	87.88		

Table 17 Limit of quantitation of succinylated zidovudine.

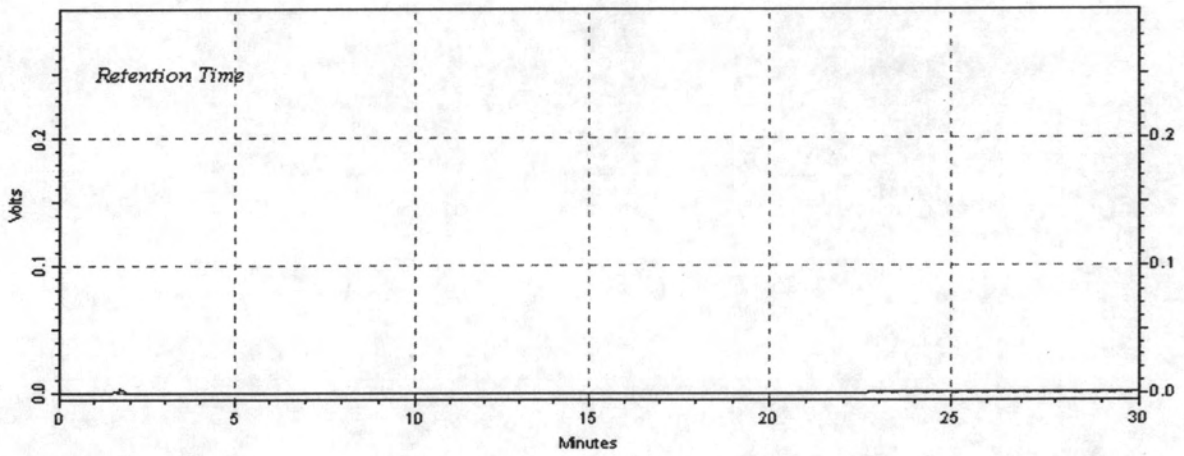
Samples	Actual Conc. (μg/mL)	Observed Conc. (μg/mL)	% Recovery	Average % recovery	% CV
A	0.107	0.115	107.57	111.58	6.98
B	0.107	0.121	112.93		
C	0.107	0.136	126.55		
D	0.107	0.118	110.16		
E	0.107	0.114	106.14		
F	0.107	0.114	106.14		

3.5 Specificity

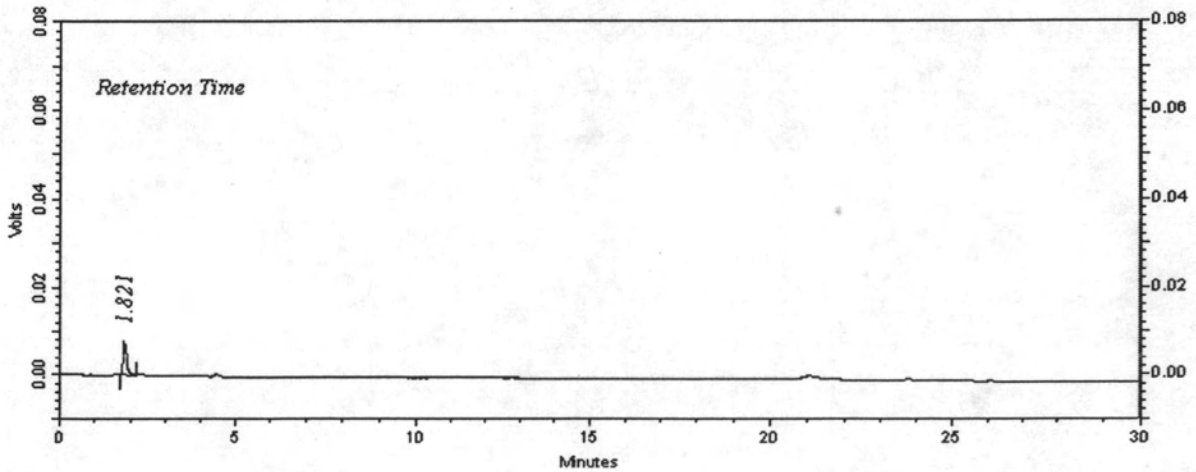
Since the drug release study was performed in buffer, it is needed to ensure that the peak of buffer would not disturb the peak of zidovudine and that of succinylated zidovudine. The peaks of buffers did not overlap the peaks of zidovudine and succinylated zidovudine. Also, the peak of the dextrin-zidovudine conjugate did not interfere with the peaks of zidovudine and succinylated zidovudine (figure 23). The results indicated the specificity of analytical method.



(A)



(B)



(C)

Figure 23 HPLC chromatograms of buffer pH 5.5 (A), buffer pH 7.4 (B), dextrin-zidovudine conjugate (C).

4. HPLC analysis in plasma

The HPLC condition for quantification of zidovudine and succinylated zidovudine in plasma was validated. Typical HPLC chromatogram of stavudine (internal standard), zidovudine and succinylated zidovudine in plasma is shown in figure 24.

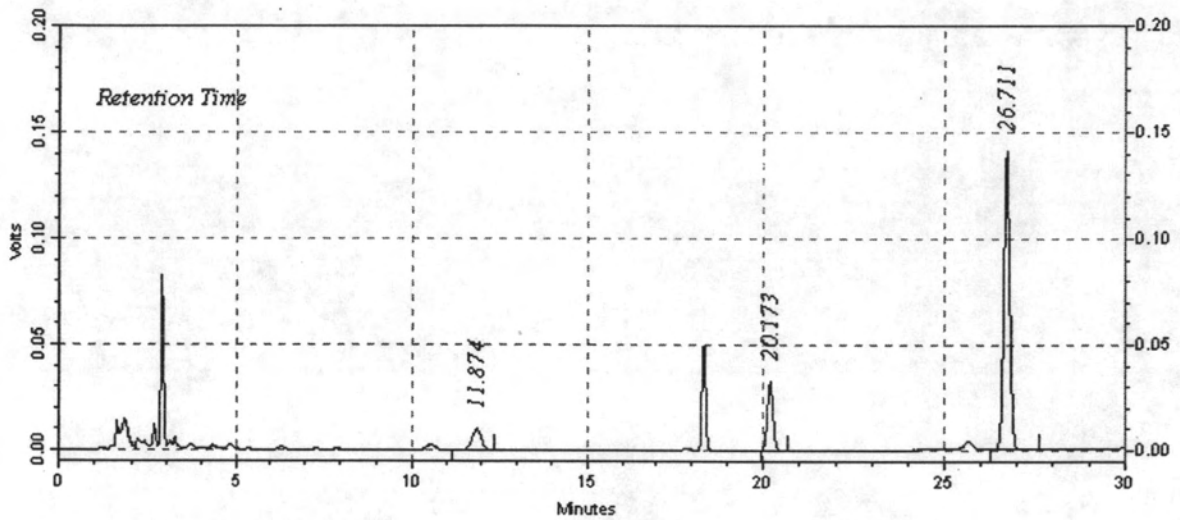


Figure 24 HPLC chromatogram of stavudine internal standard, zidovudine, and succinylated zidovudine in plasma.

Retention time of Stavudine	=	11.874	min
Retention time of Zidovudine	=	20.173	min
Retention time of Succinylated zidovudine	=	26.711	min

The standard curve of zidovudine in plasma was constructed by plotting the ratio of area under the peak of zidovudine to that of stavudine against various concentrations of zidovudine. The standard curve of zidovudine in plasma is shown in figure 25. The standard curve of succinylated zidovudine in plasma was also constructed the same as zidovudine. The standard curve of succinylated zidovudine is shown in figure 26.

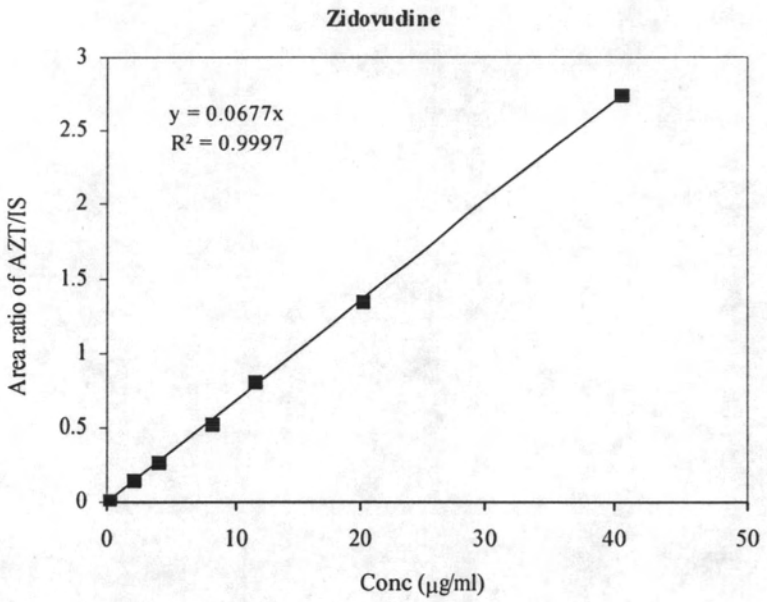


Figure 25 Standard curve of zidovudine in plasma.

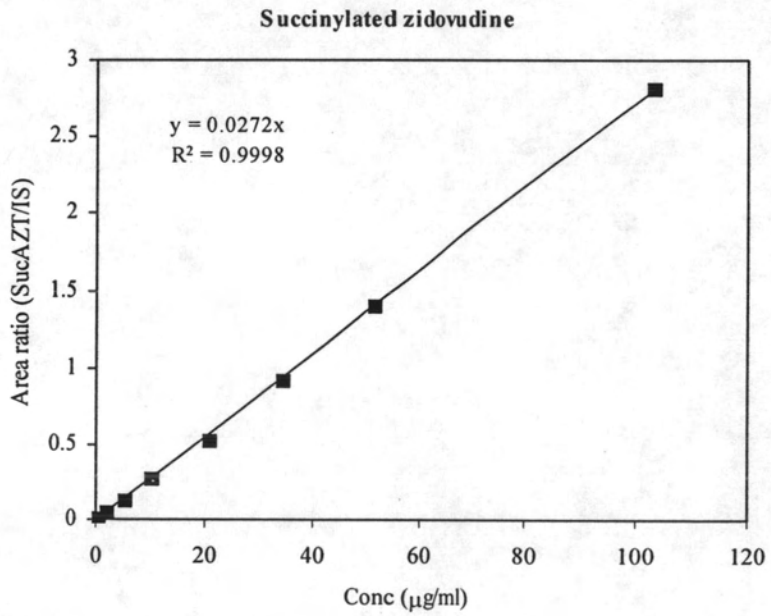


Figure 26 Standard curve of succinylated zidovudine in plasma.

4.1 Accuracy

The determination of accuracy for analytical method of zidovudine and succinylated zidovudine at three different concentrations in plasma was processed. The results are shown in tables 18 and 19, respectively.

From table 18, the % recoveries of zidovudine at individual concentration were 94.12, 102.84 and 100.92 % and the % CV of zidovudine were 1.96, 0.11 and 1.41 %, respectively.

From table 19, the % recoveries of succinylated zidovudine at individual concentration were 105.67, 100.52 and 100.21 % and the % CV of succinylated zidovudine were 0.27, 0.32 and 0.24 %, respectively.

4.2 Precision

4.2.1 Intraday precision

The determination of precision for analytical method of zidovudine and succinylated zidovudine in plasma was processed within one day by analysis of six replicates of three different concentrations. The results are shown in tables 20 and 21, respectively.

From table 20, the % recoveries of zidovudine at individual concentration were 93.37, 102.81 and 103.92 % and % CV were 0.46, 0.96 and 2.46 %, respectively.

From table 21, the % recoveries of succinylated zidovudine at individual concentration were 107.18, 100.13 and 101.39 % and % CV were 1.65, 0.79 and 1.30 %, respectively.

4.2.2 Interday precision

The determination of precision for analytical method of zidovudine and succinylated zidovudine in plasma was processed in different three days by analysis of three replicates of three different concentrations. The results are shown in tables 22 and 23, respectively.

From table 22, the % recoveries of zidovudine at individual concentration were 94.91, 99.20 and 104.04 % and % CV were 1.57, 2.10 and 3.19 %, respectively.

From table 23, the % recoveries of succinylated zidovudine at individual concentration were 109.02, 99.90 and 99.68 % and % CV were 2.43, 1.24 and 0.94 %, respectively.

Table 18 Accuracy of zidovudine in plasma.

Samples	Actual Conc. (μg/mL)	Observed Conc. (μg/mL)	% Recovery	Average % recovery	% CV
A	6.73	6.48	96.25	94.12	1.96
B	6.73	6.26	92.96		
C	6.73	6.27	93.15		
A	13.47	13.84	102.74	102.84	0.11
B	13.47	13.87	102.96		
C	13.47	13.85	102.83		
A	40.40	40.57	100.43	100.92	1.41
B	40.40	40.32	99.80		
C	40.40	41.42	102.52		

Table 19 Accuracy of succinylated zidovudine in plasma.

Samples	Actual Conc. (μg/mL)	Observed Conc. (μg/mL)	% Recovery	Average % recovery	% CV
A	2.58	2.73	105.75	105.67	0.27
B	2.58	2.72	105.36		
C	2.58	2.73	105.91		
A	6.87	6.92	100.68	100.52	0.32
B	6.87	6.92	100.73		
C	6.87	6.88	100.15		
A	82.46	82.41	99.94	100.21	0.24
B	82.46	82.72	100.31		
C	82.46	82.78	100.38		

Table 20 Intraday Precision of zidovudine in plasma.

Samples	Actual Conc. (µg/mL)	Observed Conc. (µg/mL)	% Recovery	Average % recovery	% CV
A	6.73	6.19	91.94	93.37	0.46
B	6.73	6.26	92.96		
C	6.73	6.23	92.55		
D	6.73	6.48	96.25		
E	6.73	6.29	93.39		
F	6.73	6.27	93.15		
A	13.47	13.86	102.94	102.81	0.96
B	13.47	13.84	102.74		
C	13.47	14.04	104.22		
D	13.47	13.87	102.96		
E	13.47	13.62	101.14		
F	13.47	13.85	102.83		
A	40.40	43.17	106.86	103.92	2.46
B	40.40	40.57	100.43		
C	40.40	43.21	100.95		
D	40.40	40.32	99.80		
E	40.40	43.21	106.96		
F	40.40	41.42	102.52		

Table 21 Intraday Precision of succinylated zidovudine in plasma.

Samples	Actual Conc. (μg/mL)	Observed Conc. (μg/mL)	% Recovery	Average % recovery	% CV
A	2.58	2.83	109.64	107.18	1.65
B	2.58	2.80	108.73		
C	2.58	2.78	107.72		
D	2.58	2.73	105.75		
E	2.58	2.72	105.36		
F	2.58	2.73	105.91		
A	6.87	6.91	100.50	100.13	0.79
B	6.87	6.88	100.12		
C	6.87	6.78	98.61		
D	6.87	6.92	100.68		
E	6.87	6.92	100.73		
F	6.87	6.88	100.15		
A	82.46	82.41	99.94	101.39	1.30
B	82.46	82.72	100.31		
C	82.46	82.78	100.38		
D	82.46	84.37	102.32		
E	82.46	84.89	102.94		
F	82.46	84.46	102.43		

Table 22 Interday Precision of zidovudine in plasma.

Day	Samples	Actual Conc. (μg/mL)	Observed Conc. (μg/mL)	% Recovery	Average % recovery	% CV
Day 1	A	6.73	6.23	92.55	94.91	1.57
	B	6.73	6.27	93.15		
	C	6.73	6.29	93.39		
Day 2	D	6.73	6.45	95.80		
	E	6.73	6.47	96.15		
	F	6.73	6.50	96.59		
Day 3	G	6.73	6.44	95.62		
	H	6.73	6.46	95.98		
	I	6.73	6.39	94.93		
Day 1	A	13.47	13.86	102.94	99.20	2.10
	B	13.47	13.84	102.74		
	C	13.47	13.20	98.03		
Day 2	D	13.47	13.20	98.02		
	E	13.47	13.22	98.12		
	F	13.47	13.23	98.27		
Day 3	G	13.47	13.14	97.57		
	H	13.47	13.27	98.58		
	I	13.47	13.27	98.52		
Day1	A	40.40	40.57	100.43	104.04	3.19
	B	40.40	43.17	106.86		
	C	40.40	40.32	99.80		
Day 2	D	40.40	43.30	107.18		
	E	40.40	43.39	107.40		
	F	40.40	43.66	108.07		
Day 3	G	40.40	41.70	103.22		
	H	40.40	41.09	101.70		
	I	40.40	41.10	101.74		

Table 23 Interday Precision of succinylated zidovudine in plasma.

Day	Samples	Actual Conc. (μg/mL)	Observed Conc. (μg/mL)	% Recovery	Average % recovery	% CV
Day 1	A	2.58	2.73	105.75	109.02	2.43
	B	2.58	2.72	105.36		
	C	2.58	2.73	105.91		
Day 2	D	2.58	2.83	110.01		
	E	2.58	2.89	112.14		
	F	2.58	2.83	109.85		
Day 3	G	2.58	2.88	111.82		
	H	2.58	2.83	109.65		
	I	2.58	2.85	110.69		
Day 1	A	6.87	6.91	100.50	99.90	1.24
	B	6.87	6.88	100.12		
	C	6.87	6.78	98.61		
Day 2	D	6.87	7.03	102.34		
	E	6.87	6.82	99.18		
	F	6.87	6.76	98.30		
Day 3	G	6.87	6.89	100.29		
	H	6.87	6.82	99.18		
	I	6.87	6.91	100.59		
Day1	A	82.46	82.41	99.94	99.68	0.94
	B	82.46	82.72	100.31		
	C	82.46	82.78	100.38		
Day 2	D	82.46	82.43	99.96		
	E	82.46	82.79	100.40		
	F	82.46	83.01	100.67		
Day 3	G	82.46	80.92	98.13		
	H	82.46	81.31	98.60		
	I	82.46	81.39	98.70		

4.3 Linearity

The determination of linearity for analytical method of zidovudine and succinylated zidovudine in plasma was processed at six concentrations. The results are shown in tables 24 and 25 and figures 27 and 28, respectively.

From table 24 and figure 27, the linearity study of zidovudine showed linear equation of $y = 1.0258x - 0.1814$ with r^2 of 0.9997.

From table 25 and figure 28, the linearity study of succinylated zidovudine showed linear equation of $y = 0.9967x + 0.0175$ with r^2 of 9998.

4.4 LOQ and LOD

The limit of quantification and the limit of detection for analytical method of zidovudine and succinylated zidovudine in plasma were determined. The results of limit of quantification are shown in tables 26 and 27, respectively.

From table 26, the limit of quantification of zidovudine was 0.017 μg/mL and % CV was 7.11 %.

Limit of detection of zidovudine was 0.006 μg/mL that was the concentration that provided signal-to-noise ratio of approximately 3:1.

From table 27, the limit of quantification of succinylated zidovudine was 0.034 μg/mL and % CV was 6.17 %.

Limit of detection of succinylated zidovudine was 0.008 μg/mL that was the concentration that provided signal-to-noise ratio of approximately 3:1.

Table 24 Linearity of zidovudine in plasma.

Samples	Actual Conc. (µg/mL)	Observed Conc. (µg/mL)	Average Observed Conc. (µg/mL)
A	2.02	2.18	2.18
B	2.02	2.19	
C	2.02	2.17	
A	6.73	6.48	6.31
B	6.73	6.19	
C	6.73	6.26	
A	8.08	8.02	8.12
B	8.08	8.21	
C	8.08	8.13	
A	13.47	13.84	13.86
B	13.47	13.87	
C	13.47	13.86	
A	20.20	20.33	20.30
B	20.20	20.29	
C	20.20	20.29	
A	40.40	40.57	41.35
B	40.40	43.17	
C	40.40	40.32	

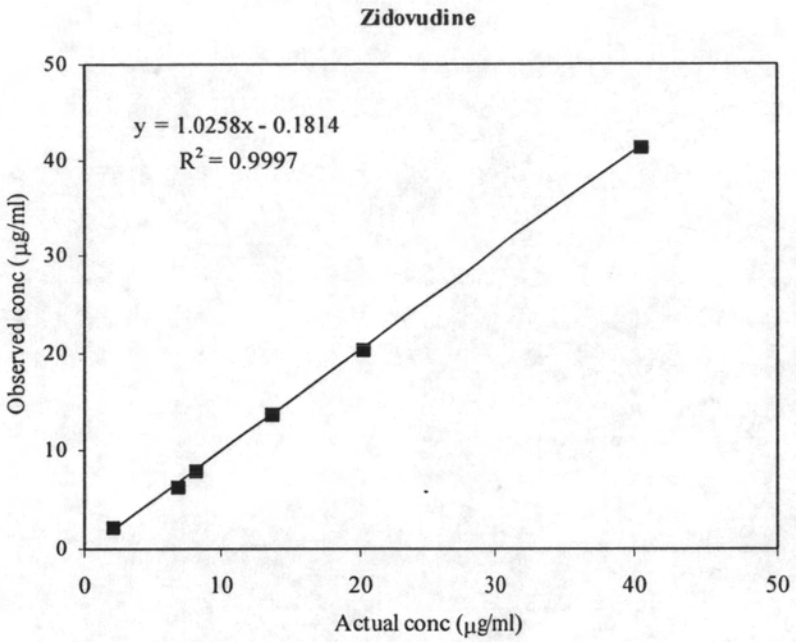


Figure 27 Linearity graph of zidovudine in plasma.

Table 25 Linearity of succinylated zidovudine in plasma.

Samples	Actual Conc. (μg/mL)	Observed Conc. (μg/mL)	Average Observed Conc. (μg/mL)
A	2.58	2.73	2.72
B	2.58	2.72	
C	2.58	2.73	
A	6.87	6.91	6.85
B	6.87	6.88	
C	6.87	6.78	
A	34.36	34.42	34.63
B	34.36	34.08	
C	34.36	35.38	
A	51.54	51.34	51.36
B	51.54	51.17	
C	51.54	51.56	
A	82.46	82.41	82.63
B	82.46	82.72	
C	82.46	82.78	
A	103.08	103.67	103.57
B	103.08	103.93	
C	103.08	103.11	

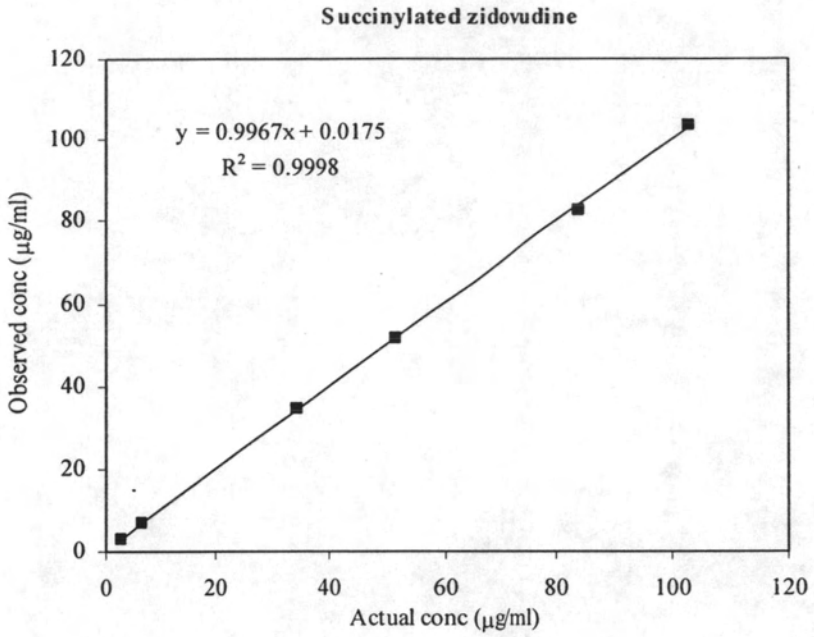


Figure 28 Linearity of succinylated zidovudine.

Table 26 Limit of quantitation of zidovudine in plasma.

Samples	Actual Conc. (μg/mL)	Observed Conc. (μg/mL)	% Recovery	Average % recovery	% CV
A	0.017	0.018	109.61	116.43	7.11
B	0.017	0.020	119.05		
C	0.017	0.020	121.73		
D	0.017	0.018	105.31		
E	0.017	0.019	114.85		
F	0.017	0.022	128.04		

Table 27 Limit of quantitation of succinylated zidovudine in plasma.

Samples	Actual Conc. (μg/mL)	Observed Conc. (μg/mL)	% Recovery	Average % recovery	% CV
A	0.034	0.040	115.18	120.04	6.17
B	0.034	0.043	125.94		
C	0.034	0.041	118.21		
D	0.034	0.045	131.65		
E	0.034	0.038	111.48		
F	0.034	0.041	117.80		

4.5 Specificity

Since the drug release study was performed in plasma, it is needed to ensure that the peak of plasma would not disturb the peak of zidovudine and that of succinylated zidovudine. The peak of plasma did not overlap the peaks of zidovudine and succinylated zidovudine indicating the specificity of analytical method as shown in figure 29.

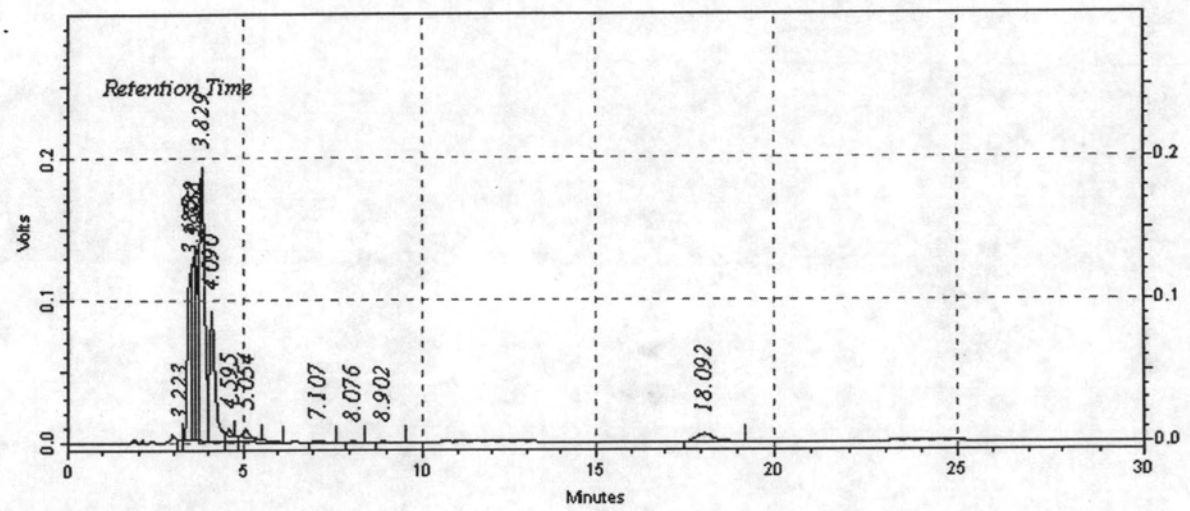


Figure 29 HPLC chromatogram of plasma.

5. *In vitro* drug release

The release of drug from the dextrin-zidovudine conjugate was performed in buffer solutions at pH 5.5 (equivalent to the pH of the endosomal compartment) and 7.4 (equivalent to that of extracellular fluids) to investigate the susceptibility to hydrolysis of the dextrin-zidovudine conjugate. The succinic spacer of the dextrin-zidovudine conjugate contained two ester bonds. One connected to zidovudine and another connected to dextrin backbone. The hydrolysis was occurred at two cleaved ester bonds on the succinic spacer resulting in the release of either free zidovudine or succinylated zidovudine as shown in figure 30. When the cleavage occurred at ester at position 1, the dextrin-zidovudine conjugate could release succinylated zidovudine. Whereas when the cleavage occurred at ester position 2, the conjugate could release zidovudine. Succinylated zidovudine could further be cleaved to zidovudine (Giammona et al., 1998).

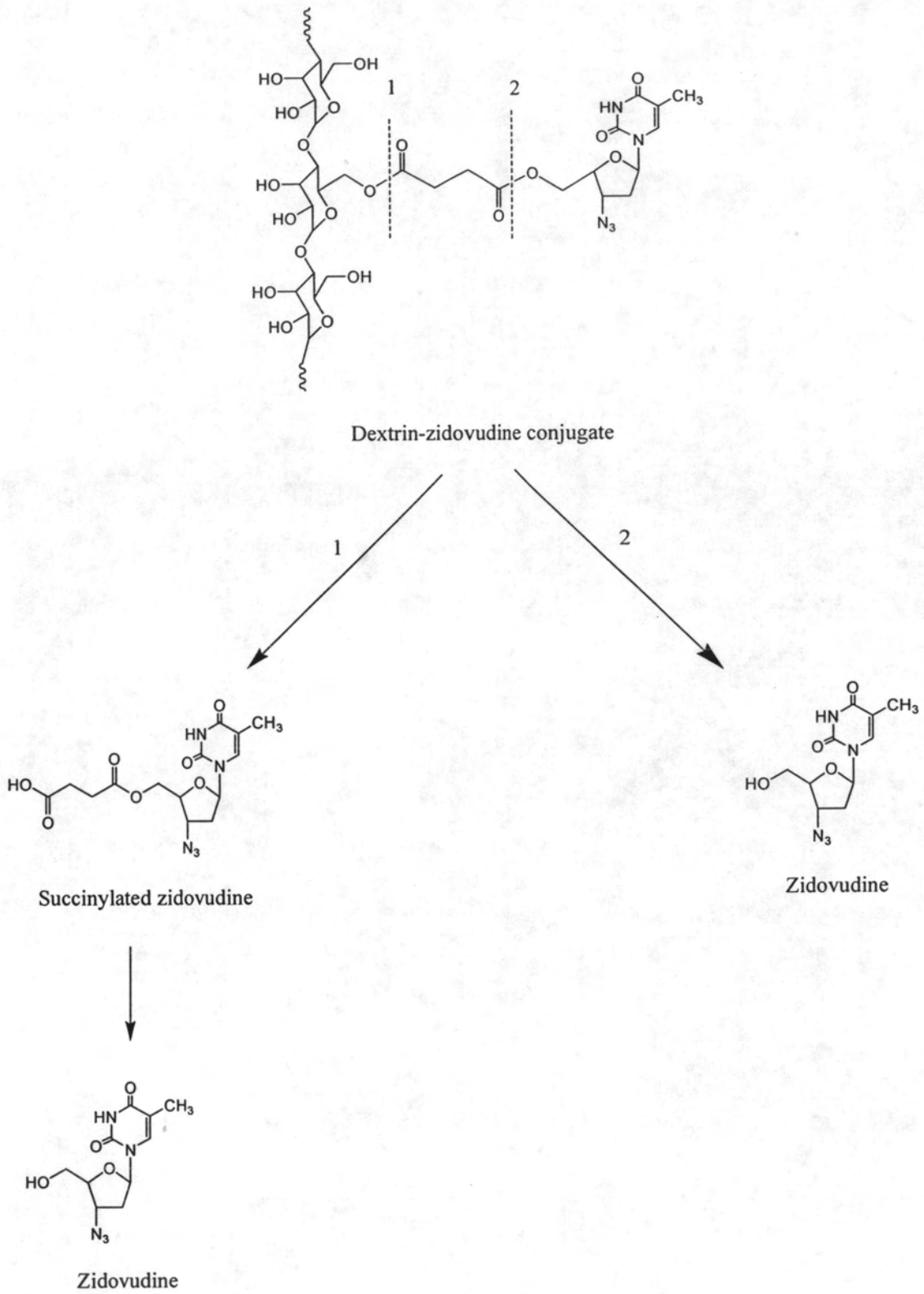


Figure 30 Ester cleavage on the succinic spacer.

5.1 Drug release study in buffer pH 5.5

The dextrin-zidovudine conjugate in buffer pH 5.5 were maintained at 37 ± 0.1 °C with continuously stirring. The sample solutions were taken out at various time intervals and the amount of zidovudine and that of succinylated zidovudine released were determined by HPLC. The results are shown in tables 31, 32, appendix II and figure 31.

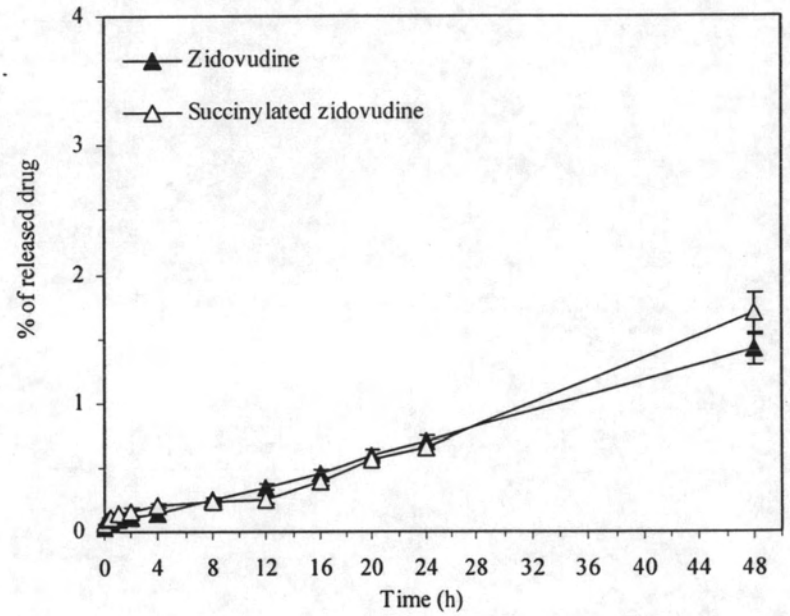


Figure 31 Release profile of the dextrin-zidovudine conjugate at pH 5.5 and 37 ± 0.1 °C (mean ± S.D., n=3).

As can be seen from figure 31, the dextrin-zidovudine conjugate could not release high amount of zidovudine and succinylated zidovudine in buffer pH 5.5. After 24 h, the release of zidovudine from the conjugate at pH 5.5 was 0.7 % and that of succinylated zidovudine was 0.7 %. After 48 h, the release of zidovudine from the conjugate was 1.4 % and that of succinylated zidovudine was 1.7 %. The starting release rate of zidovudine and succinylated zidovudine were 0.04 % /h and 0.05 %/h as calculated from equations of linear regression as shown in figure 32, respectively. The

results showed that the release of dextrin-zidovudine conjugate was very low at pH 5.5 revealing that the ester bonds were not very susceptible to hydrolysis at pH 5.5.

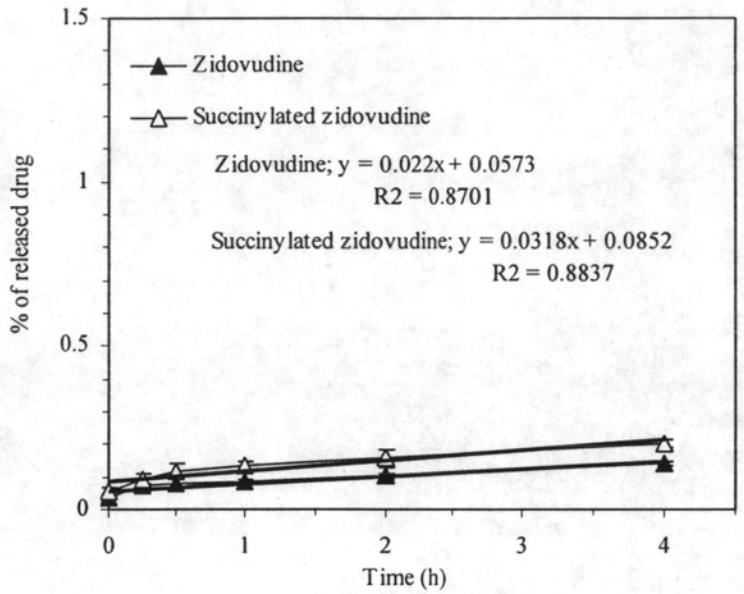


Figure 32 Linear regression of starting drug release of the dextrin-zidovudine conjugate at pH 5.5 (mean ± S.D., n=3).

5.2 Drug release study in buffer pH 7.4

The dextrin-zidovudine conjugate in buffer pH 7.4 were maintained at 37 ± 0.1 °C with continuously stirring. The sample solutions were taken out at various time intervals and the releases of zidovudine and succinylated zidovudine were determined by HPLC. The results are shown in tables 33, 34, appendix II and figure 33.

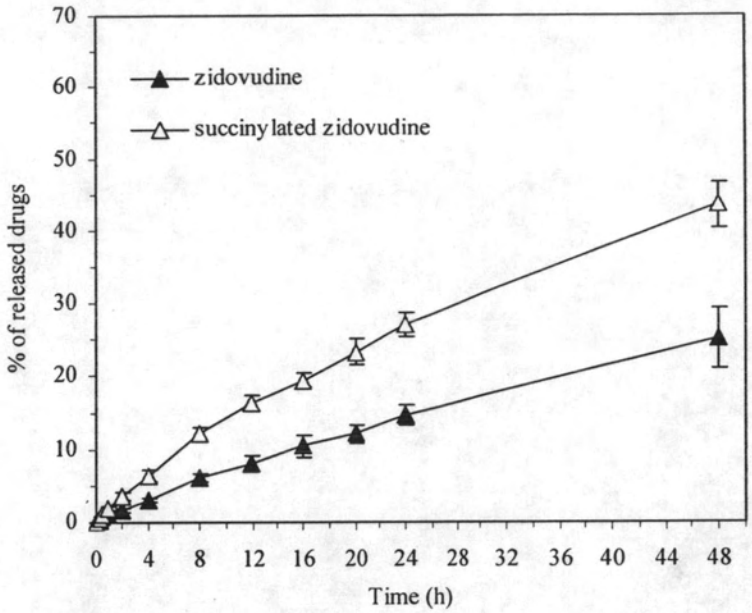


Figure 33 Release profile of the dextrin-zidovudine conjugate at pH 7.4 and 37 $\pm$ 0.1 °C (mean $\pm$ S.D., n=3).

The percentage of drug release was plotted against time as shown in figure 33. Succinylated zidovudine was released from the conjugate more than zidovudine released. After 24 h, the release of zidovudine from the conjugate at pH 7.4 was 14.7 % and that of succinylated zidovudine was 27.0 %. The release of zidovudine from the conjugate was 25.1 % and that of succinylated zidovudine was 43.4 % after 48 h. The release of succinylated zidovudine was greater than that of zidovudine revealing that the ester bond on the succinic spacer connected to dextrin was more susceptible to hydrolysis at pH 7.4 than the one connected to zidovudine. The starting release rate of zidovudine and succinylated zidovudine were calculated from equations of linear regression shown in figure 34 and were found to be 0.79 % /h and 1.62 %/h, respectively. When the release profile of zidovudine was fitted using the least square linear regression, it was nearly linear with r^2 of 0.9885 as shown in figure 35. The release of zidovudine followed zero order release.

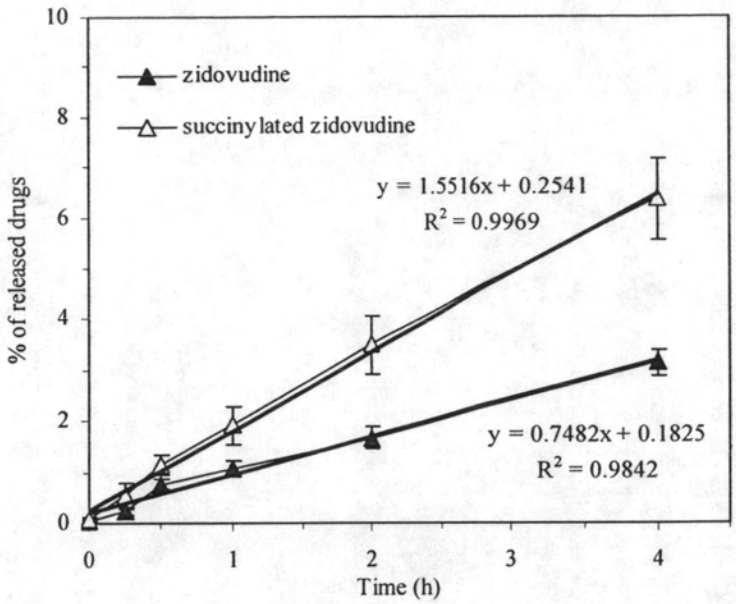


Figure 34 Linear regression of starting drug release of the dextrin-zidovudine conjugate at pH 7.4 (mean ± S.D., n=3).

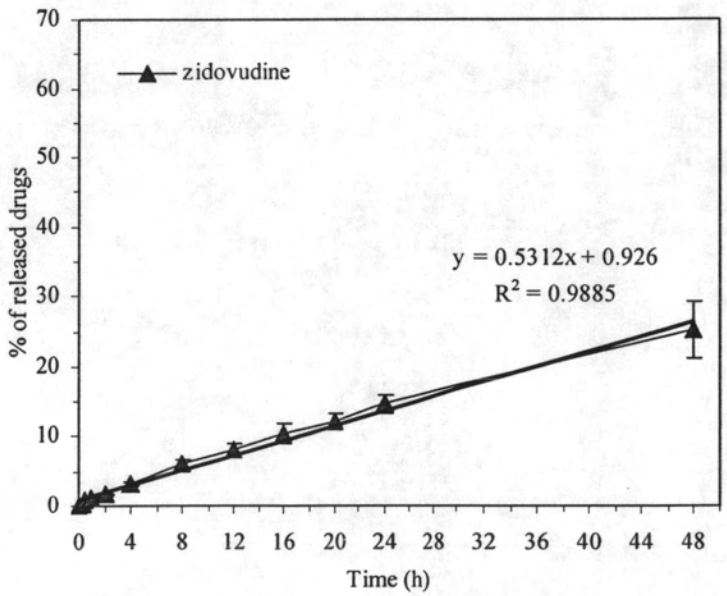


Figure 35 Linear regression of release profile of the dextrin-zidovudine conjugate at pH 7.4 (mean ± S.D., n=3).

When the release profile of the conjugate performed in buffer pH 5.5 was compared with that in buffer pH 7.4, drug release in buffer pH 7.4 was faster than that in buffer pH 5.5. It indicated that the ester bonds on succinic spacer were more susceptible to be hydrolytically cleaved in buffer pH 7.4 than in buffer pH 5.5.

From *in vitro* drug release study, the dextrin-zidovudine conjugate showed good stability at pH 5.5 and could release drug at pH 7.4.

5.3 Drug release study in plasma

The release of drug from the dextrin-zidovudine conjugate was performed in human plasma to investigate the release pattern of the dextrin-zidovudine conjugate. The dextrin-zidovudine conjugate in plasma were maintained at 37 $\pm$ 0.1 °C with continuously stirring. The sample solutions were taken out at various time intervals and the releases of zidovudine and succinylated zidovudine were determined by HPLC. The results are shown in tables 35, 36, appendix II and figure 36.

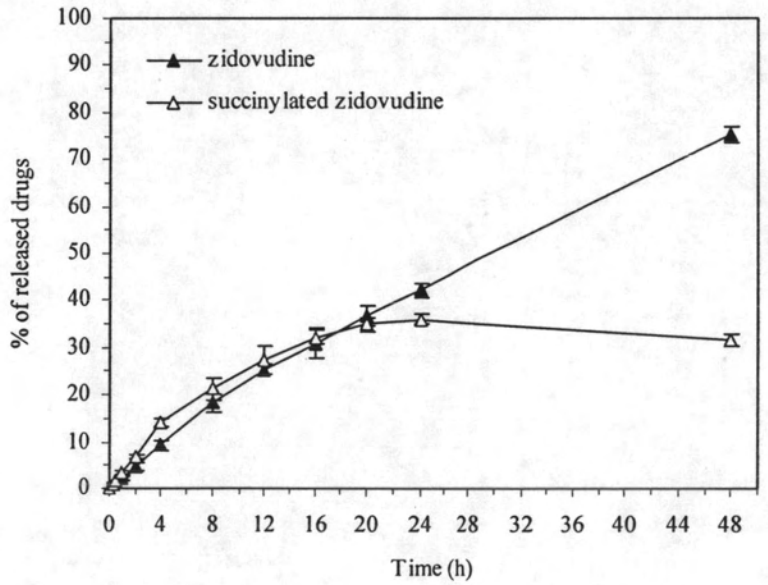
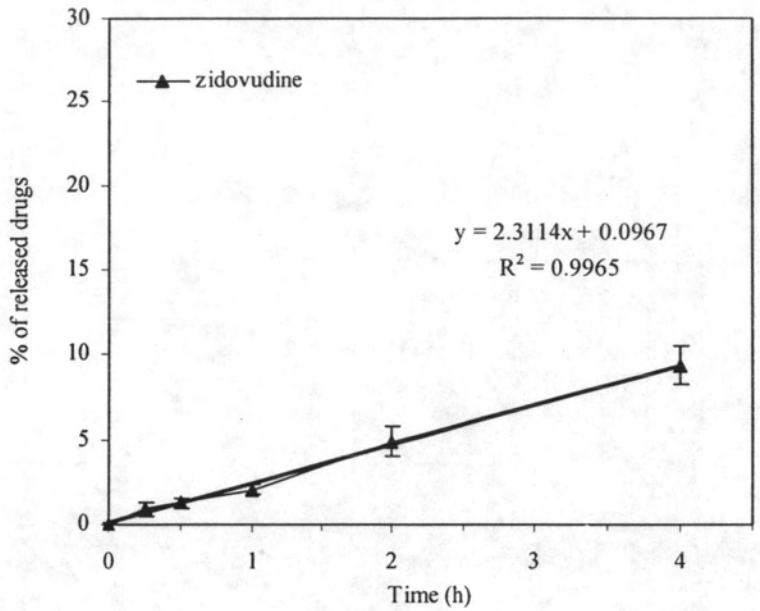


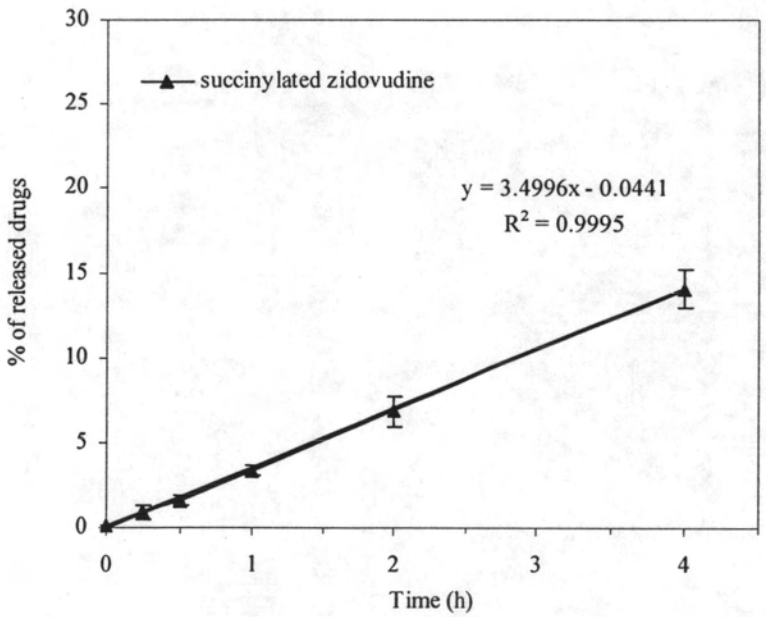
Figure 36 Release profile of the dextrin-zidovudine conjugate in plasma at 37 $\pm$ 0.1 °C (mean $\pm$ S.D., n=3).

Figure 36 showed the release of zidovudine and succinylated zidovudine from the dextrin-zidovudine conjugate in human plasma at 37 $\pm$ 0.1 °C. The amount of zidovudine released from the conjugate was greater with time. The initial release rate of zidovudine was slower than that of succinylated zidovudine. The initial release rate of zidovudine was 2.34 % /h and that of succinylated zidovudine was 3.49 % /h that were calculated from equations of linear regression shown in figure 37. The starting amount of release of succinylated zidovudine was greater than that of zidovudine. After 24 h, amount of succinylated zidovudine slightly reduced whereas that of zidovudine continued increasing because succinylated zidovudine could further be cleaved into free zidovudine. Zidovudine could be released from both dextrin-zidovudine conjugate and succinylated zidovudine. After 24 h, 42.3 % of zidovudine and 36.1 % of succinylated zidovudine were released. The dextrin-zidovudine conjugate released drug completely within 48 h (75.2 % of zidovudine released and 31.5 % of succinylated zidovudine released). When the release profile of zidovudine was fitted using the least square linear regression, it was nearly linear with r^2 of 0.9874 within 48 h as shown in figure 38. The zidovudine release in plasma followed zero order release.

When comparing the release profiles of the dextrin-zidovudine conjugate performed in human plasma with that performed in buffer pH 7.4, the release of zidovudine and succinylated zidovudine from the conjugate in plasma was faster than that in buffer pH 7.4 because plasma contains esterase enzymes that cleave ester bonds. The comparison of the results of drug release study in plasma and that in buffer pH 7.4 revealed that the succinic ester bonds were cleaved by both hydrolysis and enzymatic mechanism.



(A)



(B)

Figure 37 Linear regression of starting drug release of the dextrin-zidovudine conjugate in plasma (mean ± S.D., n=3).

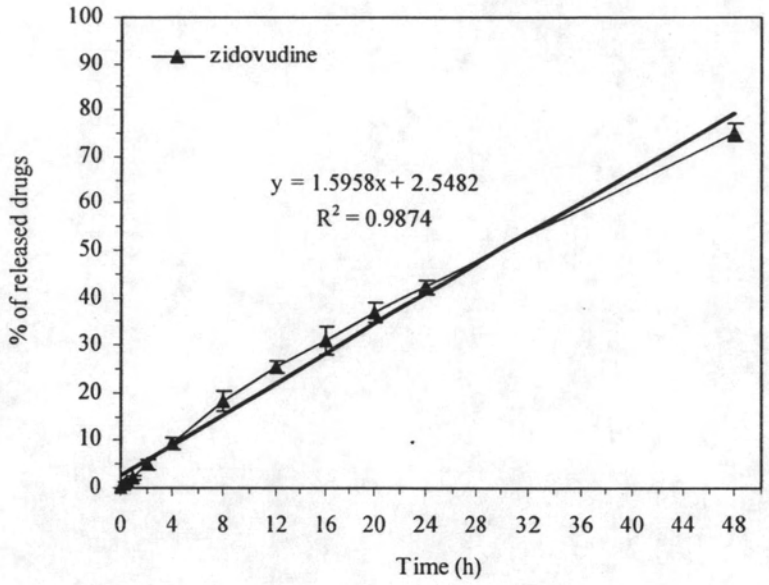
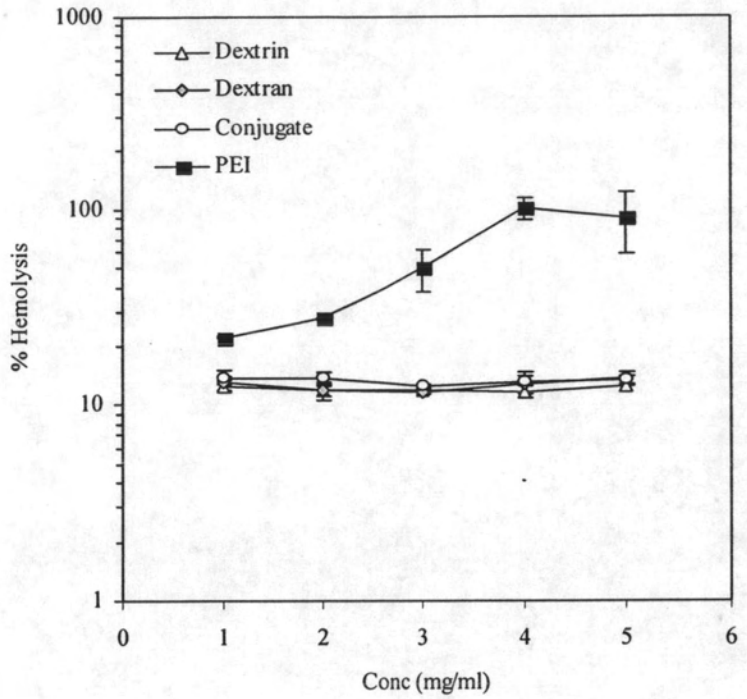


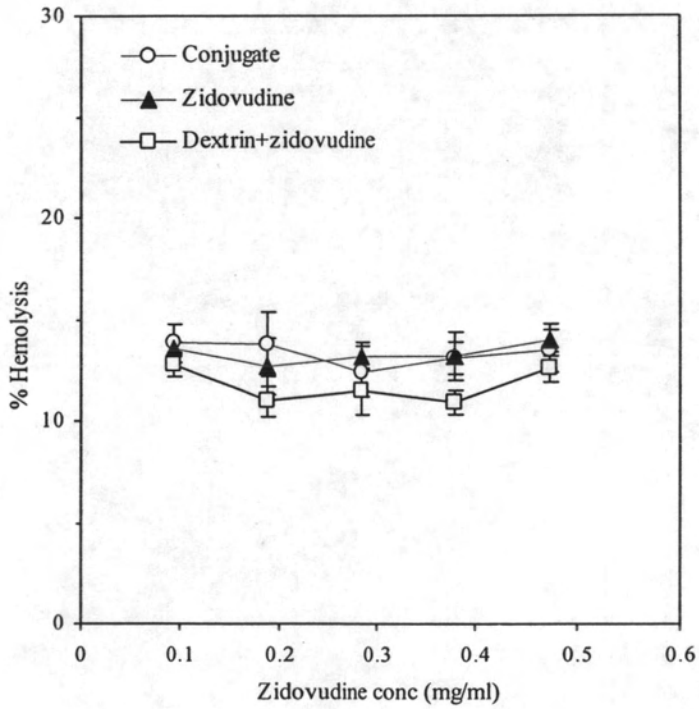
Figure 38 Linear regression of release profile of the dextrin-zidovudine conjugate in plasma (mean ± S.D., n=3).

6. Hemolysis study

To develop the dextrin-zidovudine conjugate for parenteral administration for systemic activity, the safety of the conjugate is to be studied using hemolysis test and cytotoxicity test on BEAS-2B cell lines. *In vitro* hemolysis assay is a simple and reliable method for prediction of the membrane damage *in vivo* (Jumaa et al., 2002). The degree of hemolysis obtained from the dextrin-zidovudine conjugate was compared with those of parent polymer (dextrin), dextran and poly(ethyleneimine) (PEI). Dextran is clinically used as a plasma volume expander (van Dijk-Wolthuis et al., 1997, Won and Chu, 1998) in the preparation of intravenous infusion (British Pharmacopoeia, 2002). PEI was used as positive control because it has high hemolytic activity. The hemolytic activities of dextrin, dextran, the conjugate and PEI are shown in table 37, appendix II and figure 39. Dextrin showed low haemolytic activity equivalent to dextran ($p>0.05$) whereas PEI showed high hemolytic activity ($p<0.05$). When dextrin was attached covalently to zidovudine, the dextrin-zidovudine conjugate also exhibited low hemolytic activity. Zidovudine itself showed low hemolytic activity that did not increase when it was physically mixed with dextrin or covalently linked to dextrin ($p>0.05$).



(A)

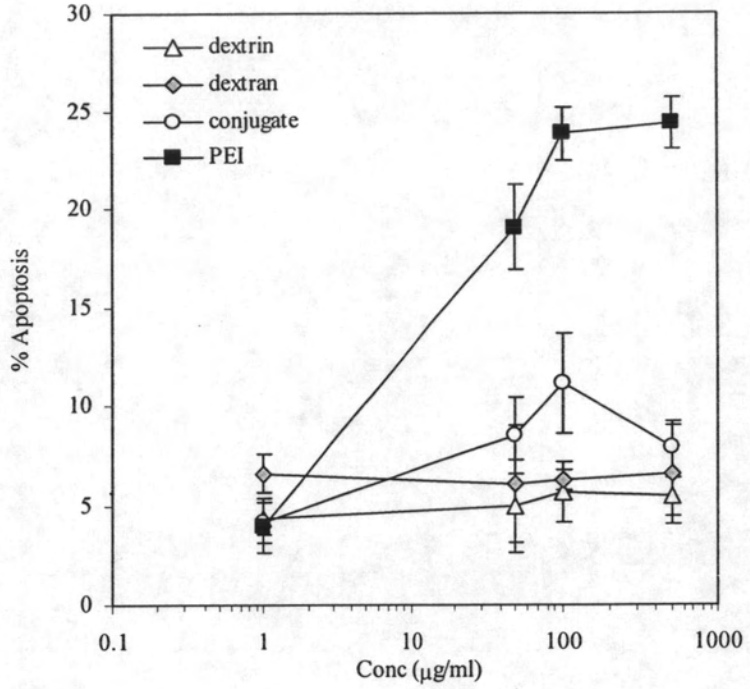


(B)

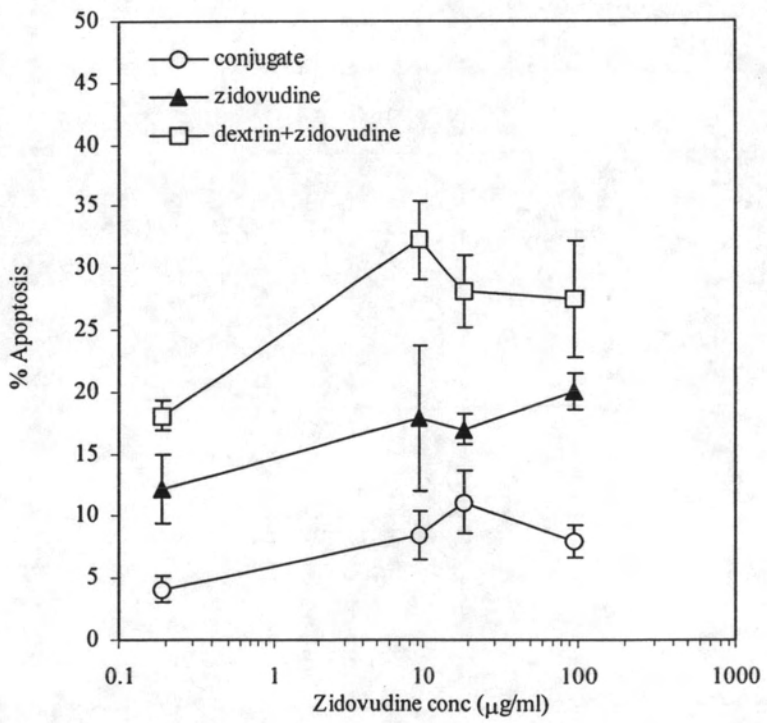
Figure 39 Hemolytic effect of dextrin, dextran, PEI and the dextrin-zidovudine conjugate (A) and those of the dextrin-zidovudine conjugate, zidovudine and combination of zidovudine and dextrin (B) (mean $\pm$ S.D., n=3).

7. Cytotoxicity study

The determination of cytotoxicity of the dextrin-zidovudine conjugate was performed using Hoechst staining assay that determine condensed chromatin caused by apoptotic cell death. Cells were incubated with different concentrations of dextrin, dextran, dextrin-zidovudine conjugate, PEI, free zidovudine and the combination of dextrin and zidovudine. Apoptosis was determined comparing between the conjugate, the combination of dextrin and zidovudine, and free zidovudine in the concentration equivalent to zidovudine. The effects of dextrin, dextran, PEI and the conjugate towards BEAS-2B cell death are shown in table 38, appendix II and figure 40A and those of the dextrin-zidovudine conjugate, the combination of dextrin and zidovudine, and free zidovudine are shown in table 38, appendix II and figure 40B. Dextrin showed low cytotoxicity similar to dextran ($p>0.05$). The conjugate showed higher toxicity than parent polymer ($p<0.05$). In the comparision to zidovudine, the conjugate showed less cytotoxicity than free zidovudine ($p<0.05$) and the physical mixture of dextrin and zidovudine that exhibited highest cytotoxicity. The cytotoxicity study on BEAS-2B cells revealed that the dextrin-zidovudine conjugate could reduce cytotoxicity of zidovudine.



(A)



(B)

Figure 40 Cytotoxicity towards BEAS-2B cells after incubation with dextrin, dextran, dextrin-zidovudine conjugate and PEI (A) and those after incubation with the dextrin-zidovudine conjugate, free zidovudine and the combination of dextrin and zidovudine (B) (mean ± S.D., n=3).

8. *In vivo* study

Free zidovudine and the dextrin-zidovudine conjugate in the dose of 8.46 mg/kg body weight (calculated as zidovudine equivalent) were administered i.v. bolus in healthy rats. The blood samples were collected at various time intervals and were analyzed by HPLC. The profiles of plasma zidovudine concentration versus time were plotted and the pharmacokinetic parameters were calculated. The individual plasma zidovudine concentration-time profiles following zidovudine administration are shown in figures 41 to 43 and those following the dextrin-zidovudine conjugate administration are shown in figures 44 to 46. The details of all data are shown in tables 39 and 40, appendix II.

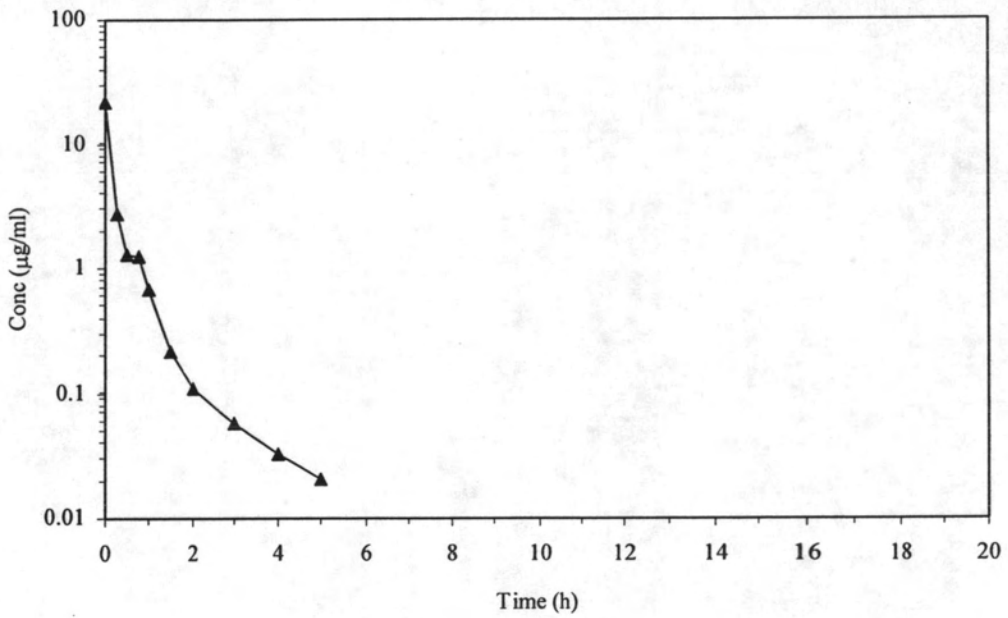


Figure 41 Zidovudine plasma concentration versus time after intravenous administration of zidovudine in rat (n1).

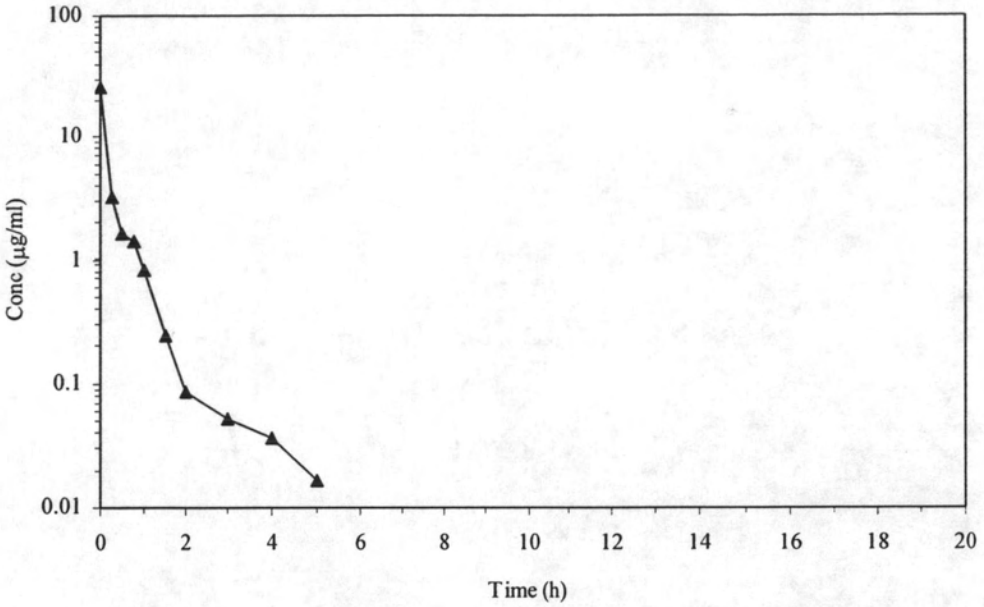


Figure 42 Zidovudine plasma concentration versus time after intravenous administration of zidovudine in rat (n2).

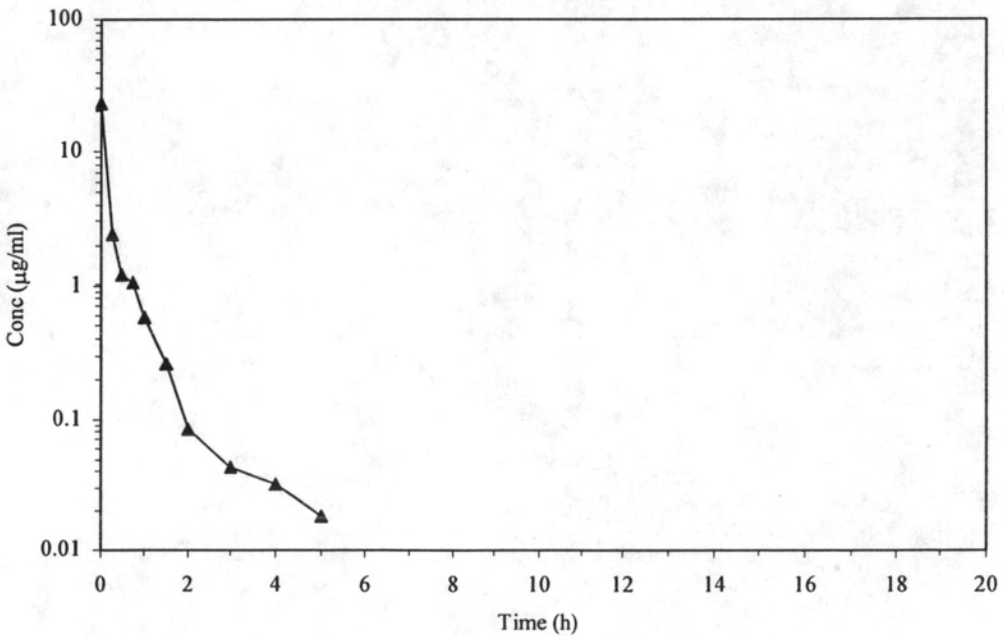


Figure 43 Zidovudine plasma concentration versus time after intravenous administration of zidovudine in rat (n3).

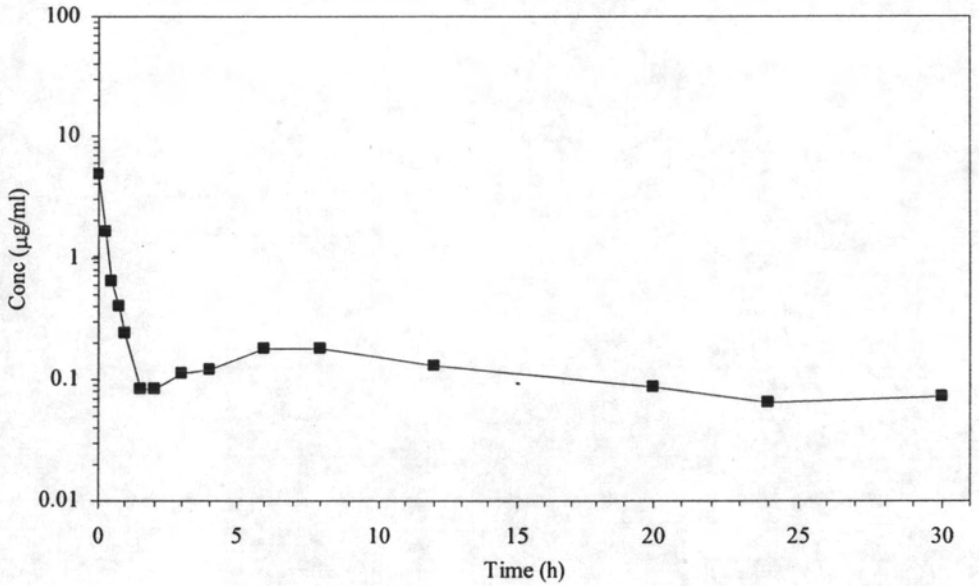


Figure 44 Zidovudine plasma concentration versus time after intravenous administration of the dextrin-zidovudine conjugate in rat (n1).

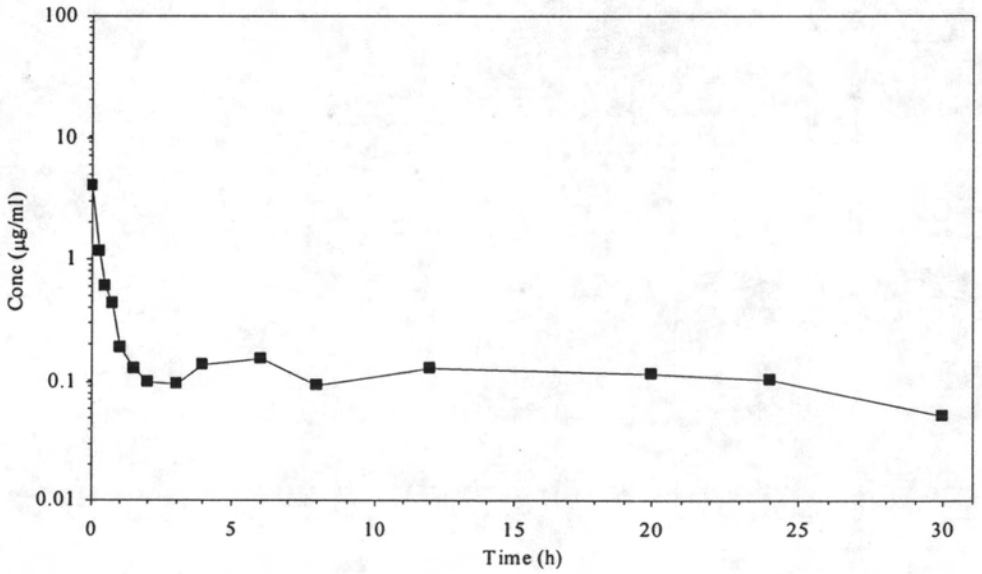


Figure 45 Zidovudine plasma concentration versus time after intravenous administration of the dextrin-zidovudine conjugate in rat (n2).

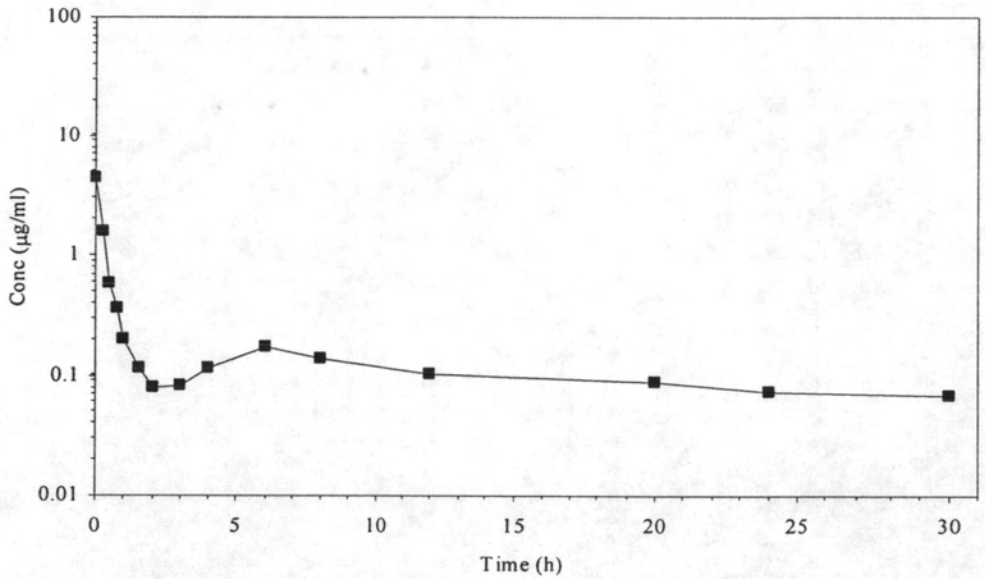


Figure 46 Zidovudine plasma concentration versus time after intravenous administration of the dextrin-zidovudine conjugate in rat (n3).

8.1 Calculation of pharmacokinetic parameters

The pharmacokinetic parameters of elimination rate constant (k_e), half-life ($t_{1/2}$), area under the plasma concentration-time curve ($AUC_{0->\infty}$), area under the first moment of the plasma concentration-time curve ($AUMC_{0->\infty}$), mean residence time (MRT), clearance (CL), apparent volume of distribution (Vd) were calculated individually.

8.1.1 Elimination rate constant (k_e)

The elimination rate constant (k_e) was directly obtained from the slope of linear regression of the terminal phase of plasma concentration-time profile that was plotted in ln-transformed scale.

The terminal phase of plasma concentration-time profile was constructed in the ln-transformed scale. The least square linear regression provided the equation that can be seen in figure 47 to 52. The elimination rate constant was obtained from slope of equation. The elimination rate constant of individual rat obtained for zidovudine were 0.56, 0.53 and 0.49 h^{-1} as shown in figures 47 to 49. The elimination rate constant of individual rat obtained for the dextrin-zidovudine conjugate were 0.05, 0.03 and 0.04 h^{-1} as shown in figures 50 to 52. The elimination rate constant obtained from the dextrin-zidovudine conjugate administration was significantly lower ($p<0.05$) than that obtained from zidovudine administration, indicating that zidovudine was eliminated from the body with slower rate when administered in the form of the dextrin-zidovudine conjugate.

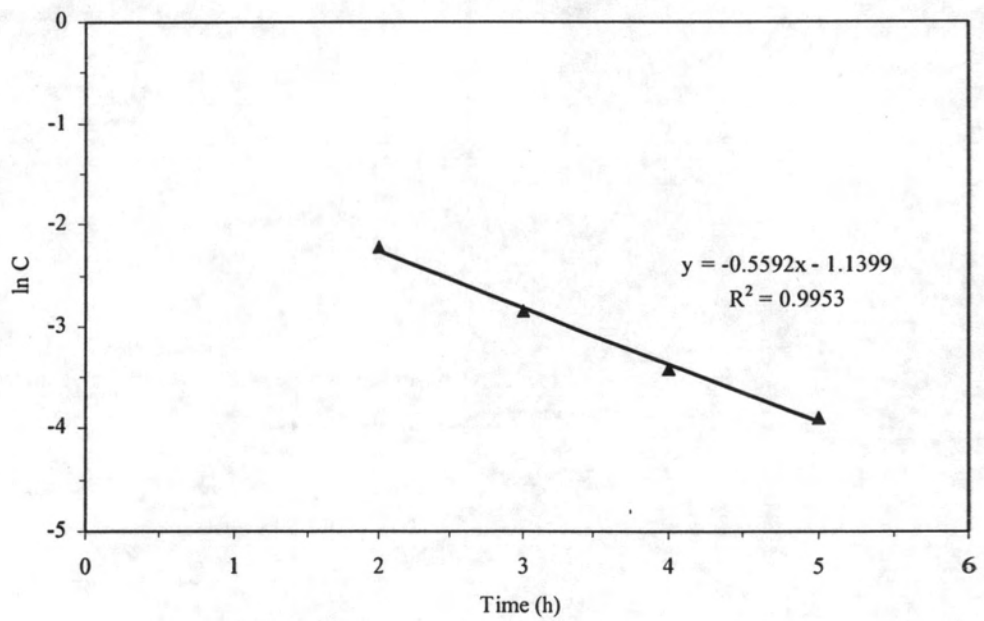


Figure 47 Linear regression of ln-transformed plasma concentration versus time after intravenous administration of zidovudine in rat (n1).

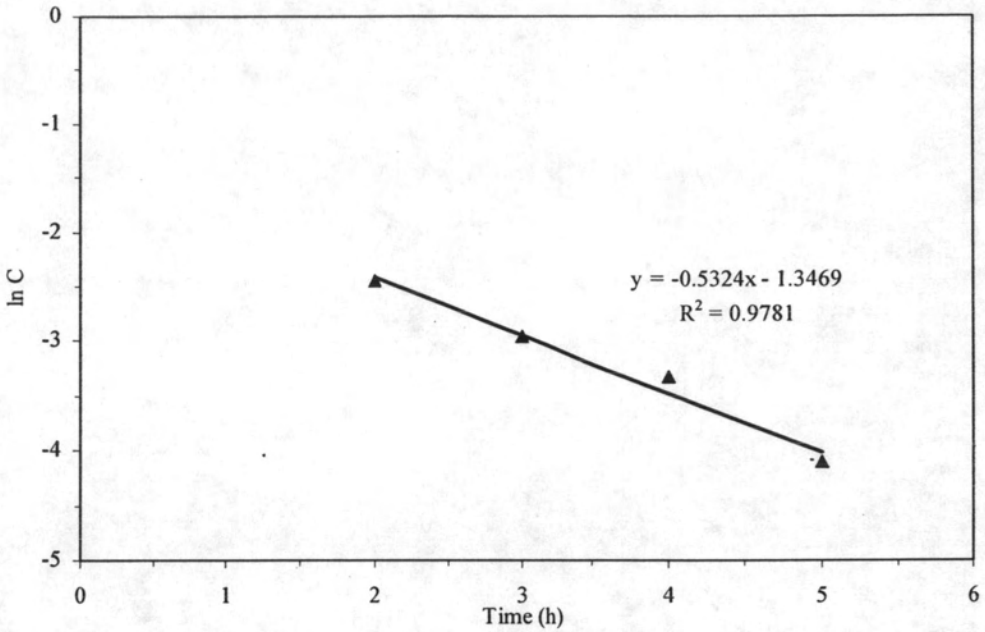


Figure 48 Linear regression of ln-transformed plasma concentration versus time after intravenous administration of zidovudine in rat (n2).

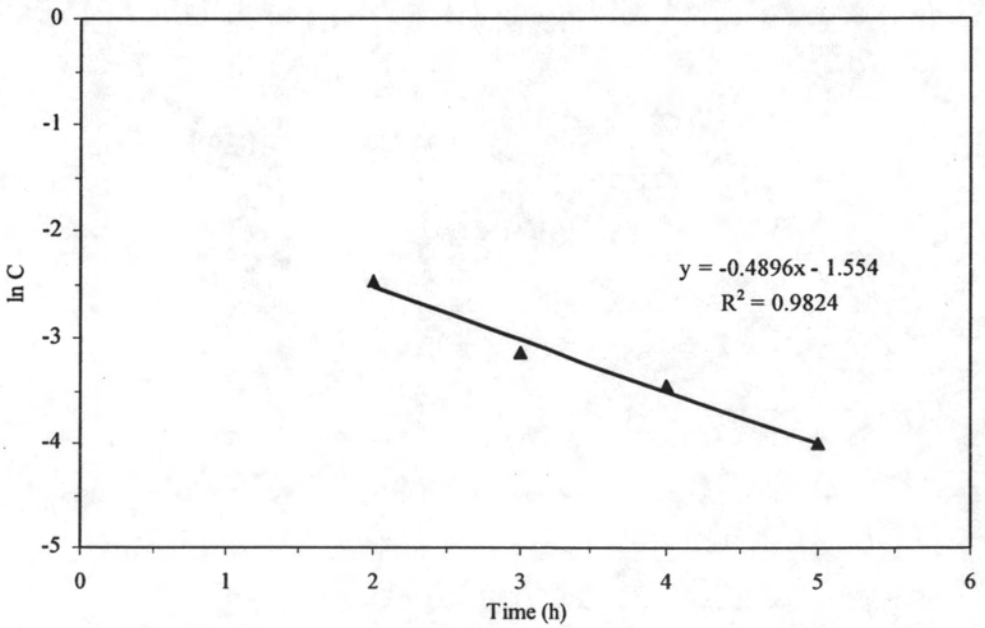


Figure 49 Linear regression of ln-transformed plasma concentration versus time after intravenous administration of zidovudine in rat (n3).

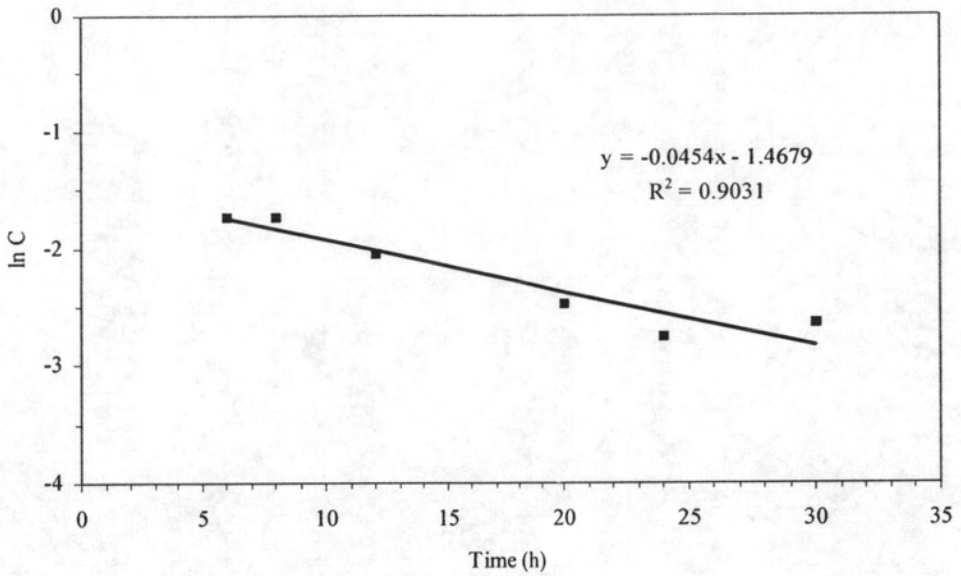


Figure 50 Linear regression of ln-transformed plasma concentration versus time after intravenous administration of the dextrin-zidovudine conjugate in rat (n1).

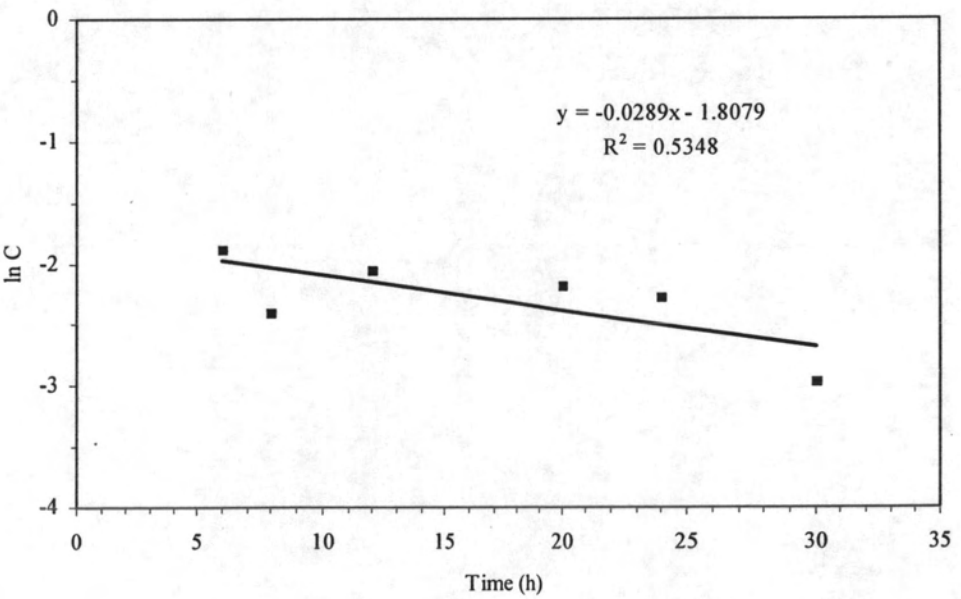


Figure 51 Linear regression of ln-transformed plasma concentration versus time after intravenous administration of the dextrin-zidovudine conjugate in rat (n2).

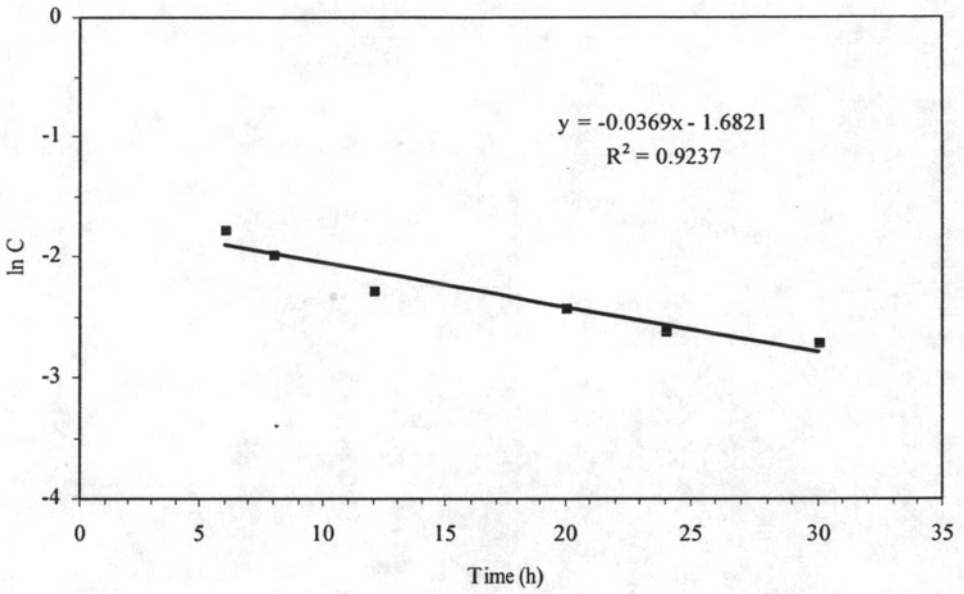


Figure 52 Linear regression of ln-transformed plasma concentration versus time after intravenous administration of the dextrin-zidovudine conjugate in rat (n3).

8.1.2 Elimination half-life ($t_{1/2}$)

Half-life is the time that the amount of drug reduces by one-half. The half-lifes of individual rat obtained for zidovudine were 1.24, 1.30 and 1.42 h as shown in table 28. The half-life of individual rat obtained for the dextrin-zidovudine conjugate were 15.26, 23.98 and 18.78 h as shown in table 29. The half-life obtained from the dextrin-zidovudine conjugate administration was significant higher ($p<0.05$) than that obtained from zidovudine administration.

8.1.3 Area under the plasma concentration time-curve ($AUC_{0->\infty}$)

$AUC_{0->\infty}$ indicates the extent of drug in systemic circulation. $AUC_{0->\infty}$ was obtained from plasma zidovudine concentration-time profile. The $AUC_{0->\infty}$ of individual rat obtained for zidovudine were 4.85, 5.70 and 4.87 μg h/mL as shown in table 28. The $AUC_{0->\infty}$ of individual rat obtained for the dextrin-

zidovudine conjugate were 6.10, 6.05 and 5.93 μg h/mL as shown in table 29. The $AUC_{0->\infty}$ obtained from the dextrin-zidovudine conjugate administration was significantly higher ($p<0.05$) than that obtained from zidovudine administration.

8.1.4 Area under the first moment of the plasma concentration-time curve ($AUMC_{0->\infty}$)

The $AUMC_{0->\infty}$ of individual rat obtained for zidovudine were 1.79, 1.89 and 1.65 μg h^2/mL as shown in table 28. The $AUMC_{0->\infty}$ of individual rat obtained for the dextrin-zidovudine conjugate were 124.18, 159.55 and 143.21 μg h^2/mL as shown in table 29.

8.1.5 Mean residence time (MRT)

Mean residence time is the average time that drug resides in the body. The MRT of individual rat obtained for zidovudine were 0.37, 0.33 and 0.34 h as shown in table 28. The MRT of individual rat obtained for the dextrin-zidovudine conjugate were 20.36, 26.37 and 24.15 h as shown in table 29. The MRT obtained from the dextrin-zidovudine conjugate administration was significant higher ($p<0.05$) than that obtained from zidovudine administration indicating that the conjugate sustained the zidovudine level in the body.

8.1.6 Apparent distribution volume (Vd)

Apparent distribution volume is the blood volume that drug dissolved. The Vd of individual rat obtained for zidovudine were 0.18, 0.14 and 0.16 l as shown in table 28. The Vd of individual rat obtained for the dextrin-zidovudine conjugate were 7.91, 10.33 and 9.65 l, as shown in table 29. The Vd obtained from the dextrin-zidovudine conjugate administration was significantly higher ($p<0.05$) than that obtained from zidovudine administration. Higher Vd of the

conjugate indicated that zidovudine from the conjugate was more concentrated in extravascular tissues than free zidovudine administration (Shargel et al., 2005).

8.1.7 Systemic clearance (CL)

Clearance (CL) is the blood volume of drug that is removed per unit time. Clearance indicates drug elimination from the body. The CL of individual rat obtained for zidovudine were 0.49, 0.42 and 0.49 l/h as shown in table 28. The CL of individual rat obtained for the dextrin-zidovudine conjugate were 0.39, 0.39 and 0.40 l/h as shown in table 29. The CL obtained from the dextrin-zidovudine conjugate administration was significant lower ($p<0.05$) than that obtained from zidovudine administration. Lower CL indicated that zidovudine from the conjugate was eliminated slower.

Table 28 Pharmacokinetic parameters of zidovudine following intravenous administration at the dose of 8.46 mg/kg in rats (n = 3).

Pharmacokinetic parameters	n1	n2	n3	Average ± S.D.
k_e (h^{-1})	0.56	0.53	0.49	0.53 ± 0.04
$t_{1/2}$ (h)	1.24	1.30	1.42	1.32 ± 0.09
$AUC_{0->\alpha}$ (μg h/mL)	4.85	5.70	4.87	5.14 ± 0.48
$AUMC_{0->\alpha}$ (μg h^2/mL)	1.79	1.89	1.65	1.78 ± 0.12
MRT (h)	0.37	0.33	0.34	0.35 ± 0.02
Vd (l)	0.18	0.14	0.16	0.16 ± 0.02
CL (l/h)	0.49	0.42	0.49	0.47 ± 0.04

Table 29 Pharmacokinetic parameters of the dextrin-zidovudine conjugate following intravenous administration at the dose of 8.46 mg/kg in rats (n = 3). The concentration is expressed as zidovudine equivalent.

Pharmacokinetic parameters	n1	n2	n3	Average ± S.D.
k_e (h^{-1})	0.05	0.03	0.04	0.04 ± 0.01
$t_{1/2}$ (h)	15.26	23.98	18.78	19.34 ± 4.39
$AUC_{0->\alpha}$ (μg h/mL)	6.10	6.05	5.93	6.09 ± 0.09
$AUMC_{0->\alpha}$ (μg h^2/mL)	124.18	159.55	143.21	142.31 ± 17.70
MRT (h)	20.36	26.37	24.15	23.63 ± 3.04
Vd (l)	7.91	10.33	9.65	9.30 ± 1.25
CL (l/h)	0.39	0.39	0.40	0.39 ± 0.01

The average pharmacokinetic parameters following the administration of free zidovudine and the conjugate are shown in table 30.

Table 30 Summary of pharmacokinetic parameters of zidovudine and of the dextrin-zidovudine conjugate following intravenous administration at the dose of 8.46 mg/kg in rats.

Pharmacokinetic parameters	Zidovudine	Dextrin-zidovudine conjugate
k_e (h^{-1})	0.53	0.04
$t_{1/2}$ (h)	1.32	19.34
$AUC_{0->\alpha}$ (μg h/mL)	5.14	6.09
$AUMC_{0->\alpha}$ (μg h^2/mL)	1.78	142.31
MRT (h)	0.35	23.63
Vd (l)	0.16	9.30
CL (l/h)	0.47	0.39

The average plasma zidovudine concentration over time after i.v. administration of the dextrin-zidovudine conjugate and free zidovudine were summarized graphically in figure 53 and table 30. After administration of free zidovudine, initial high drug concentration (24 μg/ml) was observed which decreased dramatically within 5 h. Whereas initial zidovudine level from the conjugate (4 μg/ml) was much lower than that obtained from free zidovudine administration. A more stable and higher zidovudine level was observed from the dextrin-zidovudine conjugate between 4 and 30 h compared with that from free zidovudine administration. The lower initial zidovudine level following by more stable level may reduce the fluctuation of drug level resulting in a decrease in the risk of toxicities of free drug.

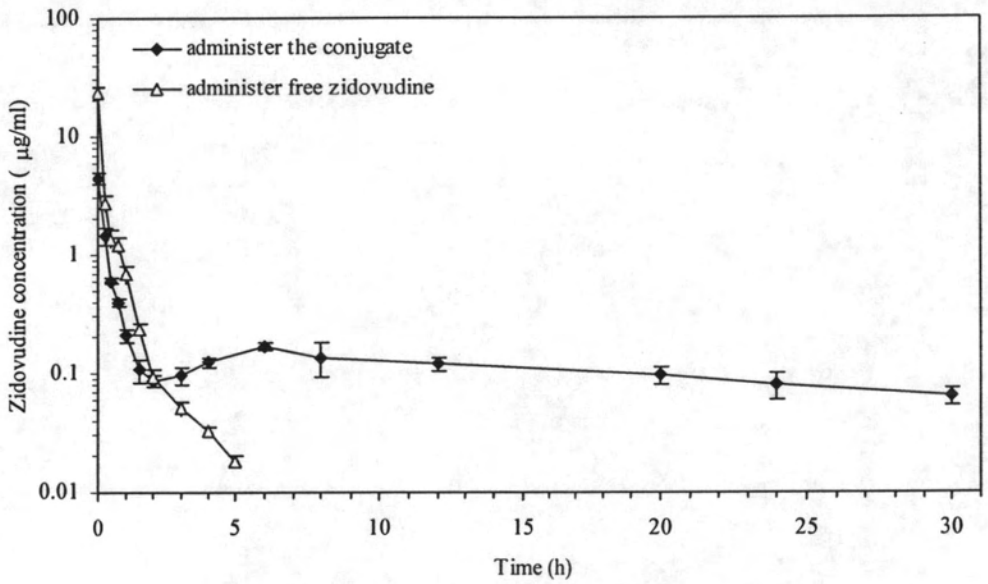


Figure 53 Average zidovudine plasma concentration versus time after intravenous administration of the dextrin-zidovudine conjugate in rats (n = 3).

The pharmacokinetic parameters for administration of the conjugate showed that the conjugate increased half-life ($t_{1/2}$), the area under the plasma concentration-time curve ($AUC_{0->\infty}$), the area under the first moment of the plasma concentration-time curve ($AUMC_{0->\infty}$), mean residence time (MRT) and

apparent distribution volume (Vd) of zidovudine. The half-life of zidovudine was increased from 1.3 h to 19.3 h after conjugation with dextrin. The increase in mean residence time from the conjugate could be resulted from decreased systemic clearance. The conjugate exhibited a reduced systemic clearance that can be explained by the higher molecular weight of the conjugate than free drug that protect the conjugate from rapid renal filtration. The *in vivo* study indicated that the dextrin-zidovudine conjugate could improve the pharmacokinetic properties of zidovudine. The dextrin-zidovudine conjugate was a potential delivery system for sustained release of zidovudine.