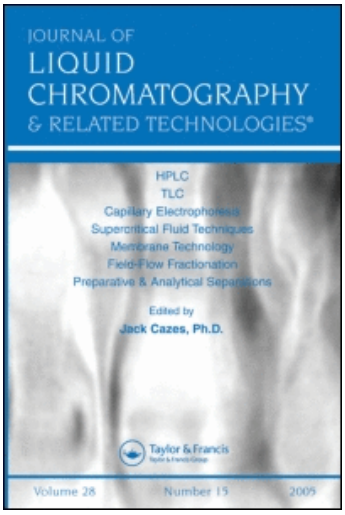

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Online publication date: 13 January 2005

To cite this Article Cserháti, Tibor , Forgács, Esther and Ságí, Gyula(1999) 'INTERACTION OF ANTIVIRAL NUCLEOSIDES WITH GAMMA CYCLODEXTRIN STUDIED BY CHARGE-TRANSFER THIN-LAYER CHROMATOGRAPHY', Journal of Liquid Chromatography & Related Technologies, 22: 1, 125 — 135
To link to this Article: DOI: 10.1081/JLC-100101648
URL: http://dx.doi.org/10.1081/JLC-100101648

J. LIQ. CHROM. & REL. TECHNOL., 22(1), 125–135 (1999)

INTERACTION OF ANTIVIRAL NUCLEOSIDES WITH GAMMA CYCLODEXTRIN STUDIED BY CHARGE-TRANSFER THIN-LAYER CHROMATOGRAPHY

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ABSTRACT

The interaction of twelve 8-substituted-2'-deoxyadenosine and seventeen 5-substituted-2'-deoxyuridine derivatives with gamma-cyclodextrin (GCD) was studied by charge-transfer chromatography carried out on reversed-phase layers and the relative strength of interaction was calculated. GCD formed inclusion complexes with the majority of drugs modifying in this manner the lipophilicity of the uncomplexed molecule. The capacity of Antiviral nucleosides to form inclusion complexes differed considerably depending on their chemical structures.

Table 1

IUPAC Names of Nucleosides

No. of Compound	IUPAC Name
1	2'-Deoxyuridine
2	Thymidine
3	2'-Deoxy-5-ethyluridine
4	2'-Deoxy-5-n-propyluridine
5	2'-Deoxy-5-isopropyluridine
6	2'-Deoxy-5-n-butyluridine
7	2'-Deoxy-5-n-pentyluridine
8	2'-Deoxy-5-n-hexyluridine
9	2'-Deoxy-5-n-heptyluridine
10	2'-Deoxy-5-n-octyluridine
11	2'-Deoxy-5-n-tetradecyluridine
12	2'-Deoxy-5-ethynyluridine
13	2'-Deoxy-5-(1-pentyn-1-yl)-uridine
14	2'-Deoxy-5-(1-hexyn-1-yl)-uridine
15	2'-Deoxy-5-(1-heptyn-1-yl)-uridine
16	2'-Deoxy-5-(1-octyn-1-yl)-uridine
17	2'-Deoxy-5-(1-decy-1-yl)-uridine
18	2'-Deoxyadenosine
19	2'-Deoxy-8-ethyladenosine
20	2'-Deoxy-8-n-propyladenosine
21	2'-Deoxy-8-n-pentyladenosine
22	2'-Deoxy-8-n-heptyladenosine
23	(Z)-2'-Deoxy-8-(propen-1-yl)-adenosine
24	(Z)-2'-Deoxy-8-(1-penten-1-yl)-adenosine
25	(Z)-2'-Deoxy-8-(1-hepten-1-yl)-adenosine
26	2'-Deoxy-8-ethynyladenosine
27	2'-Deoxy-8-(propyn-1-yl)-adenosine
28	2'-Deoxy-8-(1-pentyn-1-yl)-adenosine
29	2'-Deoxy-8-(1-heptyn-1-yl)-adenosine

INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides which can form inclusion complexes with a wide variety of inorganic and organic compounds.[1] Due to their favorable physicochemical characteristics, various CDs and CD derivatives have been frequently used in pharmaceutical formulations. CD complexation enhanced the stability of peptides in nasal enzymic systems,[2] modified the degradation of cortisone acetate and estradiol benzoate in aqueous solution[3]

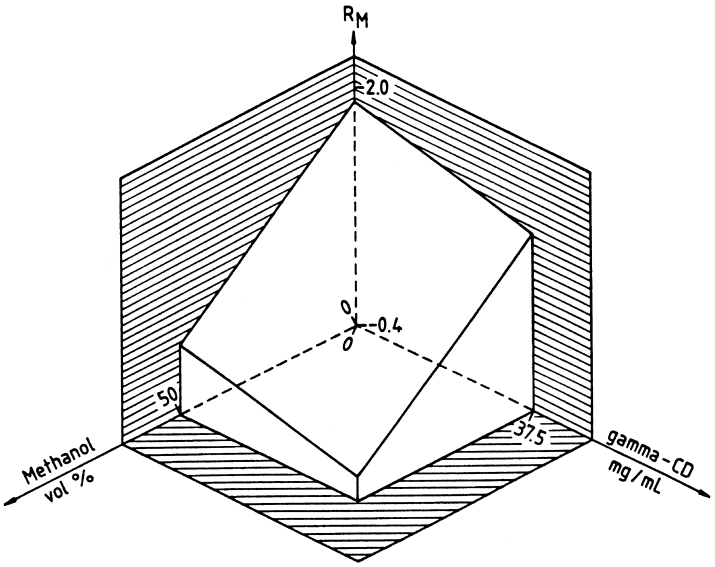

Figure 1. Effect of methanol and γ-cyclodextrin concentrations on the R_M value of nucleoside 7.

and the release of hydrocortisone from ointments,[4] improved the delivery of acitrecin through hairless mouse skin,[5] enhanced penetration of hydrocortisone into excised human skin,[6] decreased the irritation potential of pilocarpine prodrug,[7] enhanced the solubility of sparingly soluble drugs,[8] etc.

Many methods have been developed and successfully employed for the study of the formation of inclusion complexes, such as spectrophotometry,[9] thermogravimetry,[10] NMR,[11] calorimetry,[12] and freezing point depression.[13]

Various chromatographic methods, such as high-performance liquid chromatography,[14] free solution capillary electrophoresis,[15] gas-liquid chromatography,[16] and reversed-phase thin-layer chromatography (RP-TLC),[17] have also been used for the study of the interaction of CDs with drugs.

Due to its considerable practical and theoretical importance, many compounds were tested as carriers for Antiviral nucleotides. The use of an amphiphilic peptide,[18] cationic lipid particles,[19] and cationic polyhexyl-cyanoacrylate nanoparticles[20] was recently reported. CDs and CD derivatives have been also applied as carriers for oligonucleotides,[21] and it was established that the sterical and hydrophobic parameters of nucleotides govern their

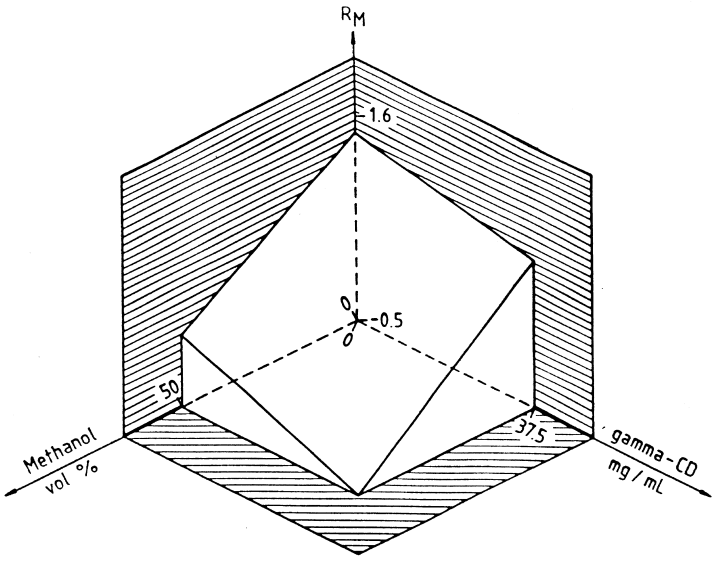

Figure 2. Effect of methanol and γ-cyclodextrin concentrations on the R_M value of nucleoside 8.

interaction with hydroxypropyl-β-cyclodextrin.[22] The objectives of the study was the determination of the interaction of some Antiviral nucleosides with gamma-cyclodextrin (GCD) and the assessment of the strength of GCD-nucleoside inclusion complexes.

EXPERIMENTAL

Reversed-phase RP-18W/UV_{254} plates (Macherey-Nagel, Dürren, Germany) were used for the determination of the relative strength of interaction without any pretreatment. Gamma-cyclodextrin (GCD) was purchased from Cyclolab Research and Development Laboratory (Budapest, Hungary) and was used as received. The IUPAC names of nucleosides are compiled in Table 1. The solutes were dissolved in methanol at a concentration of 5 mg/mL, and 4 μL of the solutions were spotted separately on the plates. As the object was the study of the interaction between nucleosides and GCD and not the elucidation of the influence of GCD on their separation, the nucleosides were separately spotted on the plates. Mobile phases were water-methanol mixtures, the methanol concentration varying between 0-80 vol.%. in steps of 5 vol.%. Methanol was chosen as the organic modifier because it forms only weak complexes with CDs.[23,24]

Table 2

Parameters of Linear Correlations Between the Lipophilicity (R_M) of Antiviral Nucleosides 1 - 10 and the Methanol (C_1 vol.-%) and γ-Cyclodextrin (C_2 mg/mL) Concentrations in the Mobile Phase*

$$R_M = R_{M0} + b_1.C_1 + b_2.C_2$$

Parameter	No. of Antiviral Nucleosides				
	12	**13**	**14**	**15**	**16**
n^a	12	15	14	15	15
R_{M0}	0.15	0.39	0.69	0.80	0.83
$-10^2 x b_1$	4.07	3.26	3.70	2.88	3.04
$10^3 x s_{b1}{}^b$	0.33	0.31	0.34	0.22	0.20
$-10^2 x b_2$	0.71	0.44	0.68	0.70	0.78
$10^3 x s_{b2}{}^b$	0.19	0.20	0.22	0.18	0.16
b'_1 (%)^c	76.44	82.90	77.90	76.77	75.58
b'_2 (%)^c	23.56	17.10	22.10	23.23	24.42
r^{2d}	0.9432	0.9093	0.9142	0.9339	0.9859
$F_{calc.}{}^e$	74.76	60.15	58.64	84.80	116.20
	6	**7**	**8**	**9**	**10**
n^a	17	14	15	16	21
R_{M0}	1.09	1.45	1.85	2.15	2.63
$-10^2 x b_1$	2.80	2.93	3.09	3.20	3.80
$10^3 x s_{b1}{}^b$	0.09	0.14	0.13	0.19	0.17
$-10^2 x b_2$	1.15	1.14	1.23	1.14	0.60
$10^3 x s_{b2}{}^b$	0.09	0.19	0.21	0.23	0.19
b'_1 (%)^c	71.07	77.65	80.73	76.51	87.65
b'_2 (%)^c	28.93	22.35	19.27	23.49	12.35
r^{2d}	0.9859	0.9746	0.9800	0.9613	0.9710
$F_{calc.}{}^e$	490.99	210.79	293.37	161.39	300.91

* Numbers refer to Antiviral nucleosides in Table 1. b_1 = decrease in the R_M value caused by 1% increase in methanol concentration in the eluent (related to the specific hydrophobic surface area of Antiviral nucleosides; b_2 = decrease in the R_M value caused by 1 mg/mL concentration change of GCD in the eluent (related to the relative strength of interaction); [a]Number of data points; [b]Standard deviations of b_1 and b_2; [c]Standard partial regression coefficients of b_1 and b_2, which are normalized to unity; [d]Coefficient of determination; [e]Calculated F value indicating the fitness of Eq.[2] to the experimental data.

The concentration of GCD in the mobile phase was 0, 25, and 50 mg/mL. Developments were carried out in sandwich chambers (22x22x3 cm) at room temperature, with the distance of development at about 16 cm. After development, the plates were dried at 105°C, and the spots of solutes were revealed by their UV absorption spectra. Each experiment was run in quadruplicate. The R_M value characterizing the molecular hydrophobicity in reversed-phase thin-layer chromatography was calculated for each solute in each mobile phase:

$$R_M = \log(1/R_f - 1) \quad (1)$$

When the coefficient of variation of the parallel determinations was higher than 5%, the R_M value was omitted from the following calculations. To separate the effects of methanol and GCD on the lipophilicity of the nucleosides, the following equation was fitted to the experimental data:

$$R_M = R_{M0} + b_1.C_1 + b_2.C_2 \quad (2)$$

where R_M = R_M value for a nucleoside determined at given methanol and GCD concentrations; R_{M0} = R_M value extrapolated to zero methanol and GCD concentrations; b_1 = decrease in the R_M value caused by a 1% increase in the methanol concentration in the eluent (related to the specific hydrophobic surface area of the nucleosides);[25] b_2 = decrease in the R_M value caused by a 1 mg/mL concentration change of GCD in the eluent (related to the relative strength of interaction); and C_1 and C_2 = concentrations of methanol and GCD, respectively. Eq. 2 was applied separately for each nucleoside and for each eluent system.

In order to find the physicochemical parameters of Antiviral nucleosides significantly influencing their capacity to form inclusion complexes with GCD, stepwise regression analysis was applied.[26] The relative strength of interaction (b_2) was the dependent variable, whereas the molecular lipophilicity (R_{M0}) and specific hydrophobic surface area (b_1) of eq. 2 were the independent variables.

RESULTS AND DISCUSSION

Due to its highly lipophilic character, nucleoside 11 was strongly retained on the reversed-phase layers even at the highest methanol concentrations. Therefore, its interaction with GCD cannot be determined by this method.

The simultaneous effects of methanol and GCD concentrations on the R_M values of nucleosides 7 and 8 are shown in Figs. 1 and 2. The retention of the nucleosides decreased with increasing concentration of both methanol and GCD in the mobile phase. This phenomenon suggests that the Antiviral nucleoside-GCD complex is less lipophilic than the uncomplexed guest molecule.

Table 3

Parameters of Linear Correlations Between the Lipophilicity (R_M) of Antiviral Nucleosides 12 - 29 and the Methanol (C_1 vol.-%) and γ-Cyclodextrin (C_2 mg/mL) Concentrations in the Mobile Phase*

Parameter	No. of Antiviral Nucleosides				
	12	**13**	**14**	**15**	**16**
n[a]	142	14	15	15	12
R_{M0}	0.18	1.23	1.50	1.81	2.37
-10^2xb_1	2.37	3.09	3.03	3.33	3.73
10^3xs_{b1}[b]	0.21	0.15	0.14	0.16	0.24
-10^2xb_2	-	0.86	0.90	0.73	1.75
10^3xs_{b2}[b]	-	0.21	0.24	0.27	0.28
b'_1 (%)[c]	-	82.93	84.85	88.39	71.18
b'_2 (%)[c]	-	17.07	15.15	11.61	28.82
r^{2}[d]	0.9148	0.9741	0.9742	0.9724	0.9731
$F_{calc.}$[e]	128.89	207.05	226.60	211.09	162.47
	17	**18**	**19**	**20**	**21**
n[a]	20	16	18	18	15
R_{M0}	3.01	1.00	1.63	1.72	2.01
-10^2xb_1	4.21	3.28	3.52	3.39	3.14
10^3xs_{b1}[b]	0.18	0.13	0.16	0.13	0.22
-10^2xb_2	0.47	0.75	0.89	0.67	0.62
10^3xs_{b2}[b]	0.18	0.13	0.19	0.16	0.28
b'_1 (%)[c]	90.27	81.58	82.57	85.80	86.63
b'_2 (%)[c]	9.73	18.42	17.43	14.20	13.37
r^{2}[d]	0.9768	0.9806	0.9749	0.9812	0.9601
$F_{calc.}$[e]	357.58	328.74	290.91	392.13	144.46
	22	**23**	**24**	**25**	**26**
n[a]	15	15	15	14	15
R_{M0}	2.84	2.01	2.59	1.65	1.49
-10^2xb_1	3.95	3.36	3.74	3.55	3.84
10^3xs_{b1}[b]	0.11	0.16	0.10	0.18	0.17
-10^2xb_2	0.30	0.46	0.24	0.90	0.93
10^3xs_{b2}[b]	0.10	0.20	0.09	0.29	0.28
b'_1 (%)[c]	92.34	90.34	93.35	86.27	87.37
b'_2 (%)[c]	7.66	9.66	6.65	13.73	12.63
r^{2}[d]	0.9903	0.9768	0.9919	0.9299	0.9786
$F_{calc.}$[e]	612.52	253.11	732.37	197.84	274.29

(continued)

Table 3 (continued)

Parameters of Linear Correlations Between the Lipophilicity (R_M) of Antiviral Nucleosides 12 - 29 and the Methanol (C_1 vol.-%) and γ-Cyclodextrin (C_2 mg/mL) Concentrations in the Mobile Phase*

Parameter	No. of Antiviral Nucleosides		
	27	**28**	**29**
n^a	15	13	22
R_{M0}	1.59	2.29	2.56
-10^2xb_1	3.51	4.10	3.69
$10^3xs_{b1}{}^b$	0.20	0.19	0.15
-10^2xb_2	0.85	0.48	-
$10^3xs_{b2}{}^b$	0.34	0.15	-
b'_1 (%)^c	87.26	86.99	-
b'_2 (%)^c	12.74	13.01	-
r^{2d}	0.9622	0.9782	0.9666
$F_{calc.}{}^e$	152.64	223.95	579.30

* Numbers refer to Antiviral Nucleosides in Table 1. For symbols see Table 2.

The modification of the lipophilicity of the nucleoside may result in different mobility, uptake, adsorption capacity, and decomposition rate of the active ingredient, thereby enhancing or lessening its biological efficiency. The parameters of eq. 2 are compiled in Tables 2 and 3.

Blank entries in Table 3 indicate that, in these instances, the effect of GCD on the mobility of the Antiviral nucleoside cannot be determined. Eq. 2 fits well the experimental data (see $F_{calc.}$ values), the significance level being over 99.9%. Eq. 2 accounted for 90-99% of the total variance (see r^2 values). The parameters (R_{M0}, b_1, and b_2) of eq. 2 indicate that the lipophilicity and specific hydrophobic surface area of Antiviral nucleosides, as well as their capacity to form inclusion complexes with GCD, differ considerably. In each case, the concentration of methanol had a higher influence on the mobility of the Antiviral nucleosides than the concentration of GCD. The different strengths of interaction of the various guest molecules with GCD indicates that the complexation may influence the biological efficiency of the individual nucleosides in different ways.

No significant linear relationship was found between the complex-forming capacity and hydrophobicity parameters of nucleosides. This result suggests

that the impact of hydrophobic interactive forces on complex formation is negligible. Unfortunately, the other physicochemical parameters (i.e., pK values of the various polar substructures, electron donor and/or acceptor capacity, and steric dimensions) of these compounds have not been yet determined. Therefore, they cannot be included in the calculation of the relationship between complex forming capacity and molecular parameters.

We assume that, not only the steric correspondence of the nucleoside molecules to the GCD cavity, but also their polarity, may influence complex formation. Because each nucleoside contains polar substructures, it can be supposed that they can bind to the -OH groups on the outer sphere of GCD molecules by electrostatic forces.

It can be concluded from the data that Antiviral nucleosides readily interact with GCD probably by the formation of inclusion complexes. This interaction influences the lipophilicity parameters of the guest molecules, resulting in modified pharmacokinetic and biological characteristics of the GCD-Antiviral nucleoside complexes.

ACKNOWLEDGMENT

This work was supported by the grant OTKA T 023422.

REFERENCES

1. J. Szejtli. *Cyclodextrin Technology.* Dordrecht: Kluwer Academic Publishers (1988).

2. W. J. Irwin, A. K. Dwivedi, P. A. Holbrook, M. J. Dey. *Pharm. Res*., **18**, 1698-1703 (1994).

3. T. Loftsson, B. Jonsdottir, J. Baldwinsdottir, H. Fridriksdottir. *STP Pharma Sci*., **4**, 354-358 (1994).

4. A. Preiss, W. Mehnert, K. H. Fromming. *Pharmazie*, **49**, 902-905 (1994).

5. T. Loftsson, A. M. Sigurdardottir, J. H. Olafsson. *Int. J. Pharm*., **115**, 245-254 (1995).

6. A. Preiss, W. Mehnert, K. H. Fromming. *Pharmazie*, **50**, 121-125 (1995).

7. P. Suhonen, T. Jarvinen, K. Lehmussaari, T. Reunamaki, A. Urtti. *Pharm. Res*., **12**, 529-533, (1995).

8. M. J. Habib, T. K. Ghosh, C. O. Akogyeram, B. Ahmadi. *Drug Dev. Ind. Pharm*., **21**, 1815-1820 (1995).

9. Y. L. Loukas, E. A. Vyza, A. P. Valiraki. *Analyst*, **120**, 533-538 (1995).

10. T. Ishiguro, A. Adachi, R. Matsuno. *Biotecnol. Biochem*., **59**, 5-54 (1995).

11. A. Botsi, K. Yannakopolou, B. Berly, E. Hadjoudis. *J.Org. Chem*., **68**, 4017-4023 (1995).

12. G. Castronuovo, V. Elia, D. Fessas, A. Giordano, F. Velleca. *Carbohydr. Res*., **272**, 31-40 (1995).

13. M. Suzuki, K. Ito, C. Fushimi, T. Kondo. *Chem. Pharm. Bull*., **41**, 942-945 (1993).

14. N. Sadley-Sosnowska. *J. Chromatogr. A*, **728**, 89-95 (1996).

15. P. Gareil, D. Pernin, J. P. Gramond, F. Guyon. *J.High Res. Chromatogr*., **16**, 195-197 (1993).

16. M. Jung, D. Schmalzing, V. Schurig. *J. Chromatogr*., **552**, 43-57 (1991).

17. T. Cserháti, E. Forgács. *Anal. Biochem*., **246**, 205-210 (1997).

18. C. Pichon, I. Freulon, P. Midoux, R. Mayer, M. Monsigny, A.-C. Roche. *Antiviral & Nucl. Acid Drug Deliv*., **7**, 335-343 (1997).

19. G. B. Takle, A. R. Thierry, S. M. Flynn, B. Peng, L. White, W. de Vonish, R. A. Galbraith, A. R. Goldberg, S. T. George. *Antiviral & Nucl. Acid Drug Deliv*., **7**, 177-185 (1997).

20. H. P. Zobel, J. Kreuter, D. Werner, C. R. Noe, G. Kumel, A. Zimmer. *Anti-sense & Nucl. Acid Drug. Deliv*., **7**, 483-493 (1997).

21. Q. Y. Zhao, J. Temsamani, S. Agrawal. *Antiviral Res. Dev*., **5**, 185-192 (1995).

22. T. Cserháti, E. Forgács, J. Szejtli. *Int. J. Pharm*., **141**, 1-7 (1996).

23. A. Buvári, J. Szejtli, L. Barcza. *J. Incl. Phenom*., **1**, 151-157 (1983/1984).

24. A. Harada, S. Takahashi. *Chem. Lett*., 2089-2090 (1984).

25. C. Horváth, W. Melander, I. Molnár. *J. Chromatogr*., **125**, 129-156 (1976).

26. H. Mager. *Moderne Regressionsanalyse.* Frankfurt am Main: Salle, Sauerlander (1982), pp. 135-167.

Received May 14, 1998
Accepted June 3, 1998
Manuscript 4822-TLC

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