Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 254 (2003) 189–196
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Effect of pH and sodium chloride on the strength and selectivity of the interaction of τ-cyclodextrin with some antisense nucleosides

Tibor Cserháti*, Esther Forgács

Chemical Research Center, Institute of Chemistry, Hungarian Academy of Sciences, P.O. Box 17, 1525 Budapest, Hungary

Received 5 June 2002; received in revised form 4 November 2002; accepted 7 January 2003

Abstract

The influence of pH and the concentration of sodium chloride on the strength and selectivity of the interaction of twelve 8-substituted-2′-deoxyadenosine and sixteen 5-substituted-2′-deoxyuridine derivatives with gamma-cyclodextrin (GCD) have been studied by the spectral mapping technique (SPM). The potency values of the spectral map were regarded as indicators of the capability of antisense nucleosides and GCD to interact simultaneously taking into consideration all relevant data. It has been established that the strength of interaction is highest in acetic and lowest in alkaline solutions, and the selectivities of acidic, alkaline and salt solutions are markedly different. The length of hydrophobic alkyl substituents in antisense molecules influenced both the strength and selectivity of the interaction. The character of the base structure affected only the selectivity.
© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Antisense nucleosides; Gamma-cyclodextrin; Molecular interaction

1. Introduction

Antisense nucleosides and nucleotides show manifold biological activities. Thus, they can inhibit signal systems (Allam and Renzi, 2001), modify receptor function (Taylor et al., 2001), show cytostatic and antiviral effects (Nieto et al., 2002), etc. In order to enhance the efficacy of the active ingredient in pharmaceutical preparations, a considerable number of organic and inorganic molecules were applied as adjuvants for antisense nucleosides (Ghosh and Iversen, 2000). The beneficial effect of a cationic derivative of amphotericin B (Garcia-Chaumont et al., 2000), cationic lipids (Szoka et al., 1996), cationic lipid complexes (Ferrari et al., 2002), cationic lipid particles (Takle et al., 1997), cationic polyhexylcyanoacrylate nanoparticles (Zobel et al., 1997), amphiphilic peptides (Pichon et al., 1997) and cyclodextrins (CDs) and cyclodextrin derivatives (Zhao et al., 1997) has been reported. Furthermore, the interaction of β-CD with nucleic acid monomer units (Formoso, 1973) with dinucleotide phosphates (Formoso, 1974) and with nucleic acids (Hoffman and Bock, 1970) was also reported.

A wide variety of multivariate mathematical statistical methods have been developed for the extraction of maximal information from large data matrices (Vandeginste et al., 1998). However, an overwhelming majority of methods takes into consideration simultaneously the strength and selectivity of the effect under investigation, that is, it cannot be applied when separation of the strength and selectivity of the effect is required. This difficulty may be overcome by the spectral mapping technique (SPM) (Lewi, 1976).

* Corresponding author. Tel.: +36-1-325-7900; fax: +36-1-325-7554.
E-mail address: forgacs@cric.chemres.hu (T. Cserháti).

0378-5173/03/$ – see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0378-5173(03)00031-0

The method divides the information into two matrices using the logarithm of the original data. The first one is a vector containing the potency values related to the overall effect. The second matrix (selectivity map) contains the information concerning the spectra of activity (qualitative characteristics of the effect) (Lewi, 1989). SPM firstly calculates the logarithm of the members of the original data matrix, facilitating the evaluation of the final plots in terms of log ratios. Consecutively, SPM subtracts the corresponding column-mean and row-mean from each logarithmic element of the matrix calculating potency values. The source of variation remaining in the centered data set can be evaluated graphically (selectivity map). SPM had been previously employed for the elucidation of the relationship between the chemical structure and fungicidal activity of nonionic surfactants (Oros et al., 1997), for the study of the inhibitory effect of surfactants on sunflower downy mildew (Oros et al., 1999), and for the characterization of stationary phases in HPLC (Hamoir et al., 1994). As the selectivity matrices of SPM are generally multidimensional they cannot be evaluated by traditional visual methods. The nonlinear mapping technique (NLMAP) was developed for the reduction of the dimensionality of such matrices (Sammon, 1969). NLMAP projects the points of the original multidimensional matrices on a two-dimensional plane in such a manner that the distances between the points on the plane show maximal correspondence to their distances in the multidimensional space.

The objectives of the study were the separation of the strength and selectivity of the interaction between gamma-cyclodextrin (GCD) and antisense nucleosides in neutral, acidic, alkaline and salt-containing solutions with SPM and to find the molecular characteristics of antisense nucleosides influencing the strength and selectivity of the interaction. The more profound knowledge of GCD–nucleoside interactions may facilitate the rational design of pharmaceutical formulations containing simultaneously antisense nucleosides and GCD.

2. Materials and methods

The IUPAC names of antisense nucleosides are compiled in Table 1. They were synthesized by Dr. G. Sági at the Chemical Research Center of the Hungarian Academy of Sciences (Budapest, Hungary). The values of the relative strength of interaction between antisense nucleotides and GCD in water, in salt solution and in alkaline and acidic solutions are compiled in Table 2. The principle of the determination of the relative strength of interaction based on the measurement of the retention of nucleosides in the absence and in the presence of various concentrations of GCD under reversed-phase conditions. The slope (further *b*) of the relationship between the retention of nucleosides and the concentration of GCD in the mobile was considered to be related to the strength of interaction. The *b* values are linearly correlated with the traditional *K* values of complexometry (binding constants of the nucleosides with GCD) but numerically they are not identical. The data were taken from the publications of Cserháti et al. (1999), Cserháti and Forgács (2000), and Cserháti and Forgács (2001).

Table 1
IUPAC names of nucleosides

Number 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-ethynyluridine
12	2′-Deoxy-5-(1-pentyn-1-yl)-uridine
13	2′-Deoxy-5-(1-hexyn-1-yl)-uridine
14	2′-Deoxy-5-(1-heptyn-1-yl)-uridine
15	2′-Deoxy-5-(1-octyn-1-yl)-uridine
16	2′-Deoxy-5-(1-decy-1-yl)-uridine
17	2′-Deoxyadenosine
18	2′-Deoxy-8-ethyladenosine
19	2′-Deoxy-8-*n*-propyladenosine
20	2′-Deoxy-8-*n*-pentyladenosine
21	2′-Deoxy-8-*n*-heptyladenosine
22	(*Z*)-2′-Deoxy-8-(propen-1-yl)-adenosine
23	(*Z*)-2′-Deoxy-8-(1-penten-1-yl)-adenosine
24	(*Z*)-2′-Deoxy-8-(1-hepten-1-yl)-adenosine
25	2′-Deoxy-8-ethynyladenosine
26	2′-Deoxy-8-(propyn-1-yl)-adenosine
27	2′-Deoxy-8-(1-pentyn-1-yl)-adenosine
28	2′-Deoxy-8-(1-heptyn-1-yl)-adenosine

Table 2
Relative strength of interaction between antisense nucleotides and GCD

Number of antisense nucleotide	Water	Solution		
		Acetic acid (0.16 M)	Sodium acetate (0.16 M)	Sodium chloride (0.16 M)
1	0.71	0.33	0	0
2	0.44	0.35	0.61	0
3	0.68	0.39	0.37	0
4	0.70	0.39	0.52	0.36
5	0.78	0.29	0.67	0.32
6	1.15	0.55	0.84	0.82
7	1.14	0.98	1.08	1.00
8	1.23	1.05	1.16	1.03
9	1.14	1.26	0.97	1.18
10	0.60	1.66	0.73	1.03
11	0	0	0.35	0
12	0.86	1.06	0.76	1.00
13	0.90	1.17	0.68	1.00
14	0.73	1.52	0.68	1.17
15	1.75	1.91	0.71	1.19
16	0.47	0.90	1.21	1.32
17	0.75	0.26	0.70	0.48
18	0.89	0.82	0.89	0.97
19	0.67	1.02	0.99	0.91
20	0.62	1.06	0.48	0.89
21	0.30	1.62	0.63	0.70
22	0.46	1.02	0.65	0.77
23	0.24	1.59	0.68	1.13
24	0.90	1.02	0.57	1.04
25	0.93	0.92	0.93	0.98
26	0.85	0.93	0.86	0.99
27	0.48	1.05	0.44	0.66
28	0	1.60	0.49	0.75

Numbers refer to antisense nucleotides in Table 1.

The data matrix consisted of the values of the relative strength of interaction of antisense nucleosides with GCD in water and in 0.16 M acetic acid, sodium acetate and sodium chloride with the 28 antisense nucleosides and the four solutions considered as the observations and variables, respectively. The potency values of the spectral map were considered as indicators of the capacity of antisense nucleotides and GCD to interact with each other taking into consideration all data simultaneously. In order to determine the effect of pH and salt on the strength and selectivity of the interaction we carried out the same calculations on the transpose of the original data matrix presented in Table 2. The multidimensional spectral maps were visualized by the two-dimensional NLMAP. Calculation of the two-dimensional nonlinear map of spectral characteristics was carried out to the point where the difference between the last two iterations was lower than 10^{-8}.

For the determination of physicochemical parameters of antisense nucleosides accounting for the strength and selectivity of their interaction with GCD under various experimental conditions, stepwise regression analysis (SRA) was applied (Mager, 1982). In common multivariate regression analysis, the presence of independent variables exerting no significant effect on the change of the dependent variable considerably decreases the significance level of the equation. SRA eliminates from the selected equation the dependent variables having no significant impact on the dependent variable, increasing in this manner the reliability of the calculation. SRA was carried out three times, with the potency values and the coordinates of the two-dimensional nonlinear map considered

separately as the dependent variables. The independent variables were in each instance: the presence (1) or absence (0) of uridine base; degree of saturation of the alkyl chain (0 = saturated, 1 = presence of double bond, 2 = presence of triple bond); number of carbon atoms in the alkyl chain; number of branching in the alkyl chain. The number of accepted independent variables was not limited and the acceptance limit was set to 95% significance level.

Softwares for SPM and NLMAP were prepared by Dr. Barna Bordás, Plant Protection Institute of the Hungarian Academy of Sciences (Budapest, Hungary). Algorithms for the softwares can be found in the original publications. Software for stepwise regression analysis was purchased from Compudrug Ltd. (Budapest, Hungary).

3. Results and discussion

The data in Table 2 indicate that the majority of antisense nucleosides form complexes (probably inclusion complexes) in each solution, however, the strength of interaction markedly depends on both the chemical structure of guest molecules and the pH and salt content of the solution.

The potency values (overall capacity of individual nucleosides to interact with GCD) are compiled in Table 3. Antisense nucleosides show high differences in their capacity to interact with GCD, the interaction capacity varies between 2.78 (compound **15**) and 0.17 (compound **11**) in arbitrary units. This finding indicates that the inclusion complex formation of nucleosides may exert a different degree of effect on the biological and pharmacokinetic properties (adsorption, uptake, half-life, etc.) of nucleoside–GCD complexes resulting in modified efficacy. The data further suggest that the pharmacological characteristics of nucleosides showing higher interactive capacity can be more effectively modified by GCD complexation. The impact of salt concentration and pH on the strength of nucleoside–GCD complexes also differ considerably. It may be assumed that not only the sterical correspondence of the nucleoside molecules to the hydrophobic cavity of GCD, but also their polarity may exert a marked influence on the strength of complex formation. The dissociable polar substructures of nucleosides probably bind to the hydrophilic hydroxyl groups of GCD pointing outward of the apolar cavity by electrostatic interactive forces. As the degree of dissociation of the polar substructures depends on both the concentration of sodium chloride and pH, their capacity to bind to the hydroxyl groups of GCD also depends on these environmental factors. Unfortunately, the p*K* values of these nucleosides have not been determined, and the number of mobile phases with different pH values was not sufficient for reliable calculation of the p*K* values, consequently, the correlation between the strength of interaction and the p*K* values of nucleosides could not be calculated. The results indicate

Table 3
Capacity of antisense nucleosides to interact with GCD

Number of nucleoside	Potency		
1	0.52		
2	0.70		
3	0.72		
4	0.98		
5	1.03		
6	1.68		
7	2.10		
8	2.24		
9	2.28		
10	2.01		
11	0.17		
12	1.84		
13	1.88		
14	2.05		
15	2.78		
16	1.95		
17	1.10		
18	1.78		
19	1.80		
20	1.52		
21	1.63		
22	1.45		
23	1.82		
24	1.76		
25	1.88		
26	1.81		
27	1.32		
28	1.42		
Water	Solution		
	Sodium chloride (0.16 M)	Acetic acid (0.16 M)	Sodium acetate (0.16 M)
3.85	4.10	5.05	3.71

Potency values of spectral mapping in arbitrary units. Numbers refer to antisense nucleosides in Table 1.

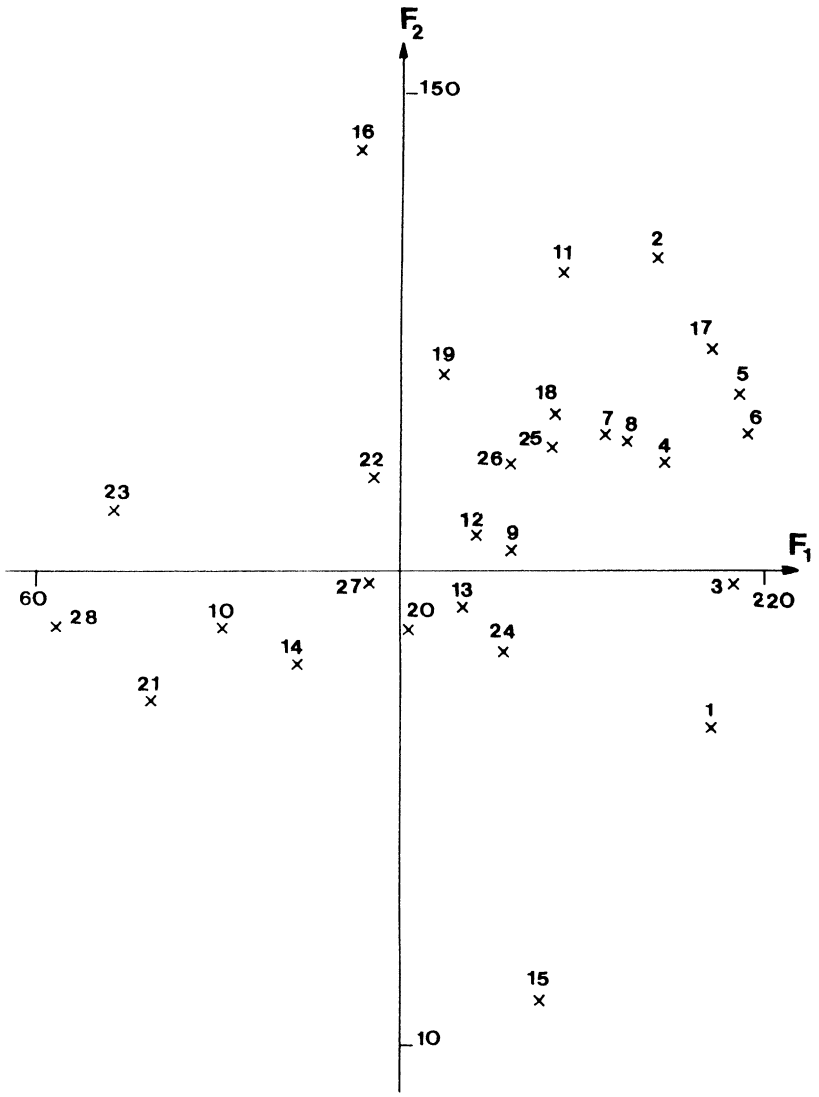

Fig. 1. Similarities and dissimilarities between the selectivity of antisense nucleosides to interact with gamma-cyclodextrin. Two-dimensional nonlinear selectivity map. Number of iterations: 43; maximal error: 6.71×10^{-3}. Numbers refer to antisense nucleosides in Table 1.

further that the effect of pH and salt concentration must be taken into consideration as an important parameter modifying the biological activity of nucleoside–GCD complexes.

The two-dimensional nonlinear selectivity map of antisense nucleosides is shown in Fig. 1. The compounds do not form clusters according to their structural characteristics (type of base, degree of saturation of the alkyl chain, number of carbon atoms in the alkyl chain, number of branching in the alkyl chain). This fact can be tentatively explained by the supposition that the impact of each structural parameter on the selectivity is commensurable and the effect observed is the result of the interplay of the various individual effects.

Solutions are widely distributed on the two-dimensional nonlinear selectivity map (Fig. 2). This scattering demonstrates that the pH and the concentration

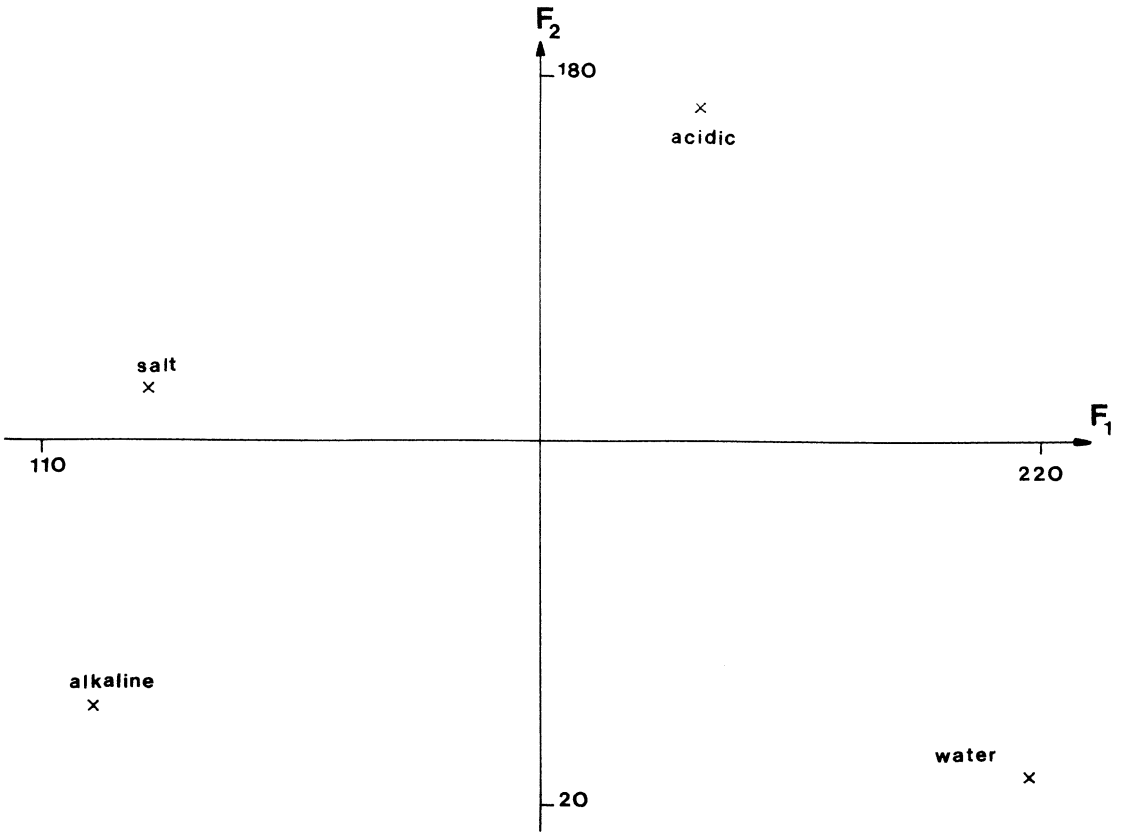

Fig. 2. Similarities and dissimilarities between the selectivity of acidic, alkaline pH and the concentration of sodium chloride. Two-dimensional nonlinear selectivity map. Number of iterations: 41; maximal error: 4.10×10^{-3}.

of sodium chloride have a different effect on both the strength and the selectivity of the interaction of antisense nucleosides with GCD.

Stepwise regression analysis pointed to significant relationships between the potency values and the first coordinate of the two-dimensional nonlinear selectivity map (SPM1) and the structural characteristics of antisense nucleosides ($n = 28$):

$$\text{Potency} = 0.88 + (0.15 \pm 0.03)\ \text{length of alkyl chain},$$
$$r_{\text{calc.}} = 0.6840, \quad r_{99.9\%} = 0.5880 \qquad (1)$$

$$\text{SPM1} = 189.0 - (12.33 \pm 1.74)\ \text{length of alkyl chain} + (45.69 \pm 8.83)\ \text{presence of uridine base},$$
$$r^2 = 0.7285, \quad F_{\text{calc.}} = 33.53, \quad F_{99.9\%} = 9.22 \qquad (2)$$

Equations fit well the experimental data the significance level being over 99.9% in both cases (compare $r_{\text{calc.}}$ and $F_{\text{calc.}}$ values with tabulated ones). The selected independent variables explain the considerable ratio of variance (Eq. (1) = 46.79%; Eq. (2) = 72.85). Eq. (1) reveals that the strength of interaction increases with the increasing length of apolar alkyl chain in the nucleoside molecule. This finding indicates that not only the bulky base structure but also the alkyl chain may enter the GCD cavity binding to the apolar wall of cavity by hydrophobic interactive forces.

Selectivity partially depended on the character of basis and on the length of alkyl chain (no significant relationship was found between the second coordinate of the two-dimensional non-linear spectral map and the structural characteristics of antisense nucleosides).

The relative impact of both independent variables were similar (basis: 42.17%; length of alkyl chain: 57.83). This result can be explained by the assumption that the dimensions of basis modify their sterical correspondence with the GCD cavity while the polarity influences the hydrophilic interactions with the polar parts of GCD. The negative effect of the length of alkyl chain on the selectivity suggests that the selectivity of binding of antisense nucleosides with shorter alkyl chain to GCD show greater differences than the binding of derivatives with longer alkyl chains.

It may be concluded from the data that the strength and selectivity of the interaction of antisense nucleosides with GCD considerably depend on the pH and sodium chloride concentration. The effect is different for different antisense nucleosides. The interaction involves the inclusion of the base structure or their hydrophobic substructures into the cavity of GCD. This phenomenon is governed by the sterical correspondence of the interacting molecules and by the hydrophobic binding forces. Polar (electrostatic) interactions (probably hydrogen bond formation) may occur between the hydroxyl groups of GCD and the substructures of nucleosides not included in the GCD cavity. These hydrophilic interactions can modify the selectivity of the binding of antisense nucleosides to GCD.

References

Allam, M., Renzi, P.M., 2001. Inhibition of GM-CSF-3/IL-5 signaling by antisense oligodeoxynucleotides targeting the common beta chain of their receptors. Antisense Nucleic Acid Drug Dev. 11, 289–300.

Cserháti, T., Forgács, E., 2000. Effect of gamma-cyclodextrin, methanol and pH on the parameters of reversed-phase thin-layer chromatography of some antisense nucleosides. J. Incl. Phenom. Macrocycl. Chem. 38, 453–465.

Cserháti, T., Forgács, E., 2001. Quantitative relationship between the chemical structure of antisense nucleosides and their capacity to interact with cyclomalto-octaose. Eur. J. Pharm. Biopharm. 51, 39–44.

Cserháti, T., Forgács, E., Sági, G., 1999. Interaction of antiviral nucleosides with gamma cyclodextrin studied by charge transfer thin-layer chromatography. J. Liq. Chromatogr. Relat. Technol. 22, 125–135.

Ferrari, M.E., Rusalov, D., Enas, J., Wheeler, C.J., 2002. Synergy between cationic lipid and co-lipid determines the macroscopic structure and transfection activity of lipoplexes. Nucleic Acid Res. 30, 1808–1816.

Formoso, C., 1973. Interaction of β-cyclodextrin with nucleic acid monomer units. Biochem. Biophys. Res. Commun. 50, 999–1005.

Formoso, C., 1974. Interaction of β-cyclodextrin with dinucleoside phosphates. Biopolymers 13, 909–917.

Garcia-Chaumont, C., Seksek, O., Jolles, B., Bolard, J., 2000. A cationic derivative of amphotericin B as a novel delivery system for antisense oligonucleotides. Antisense Nucleic Acid Drug Dev. 10, 177–184.

Ghosh, C., Iversen, P.L., 2000. Intracellular delivery strategies for antisense phosphorodiamidate. Antisense Nucleic Acid Drug Dev. 10, 263–274.

Hamoir, T., Cuaste Sanchez, F., Bourguignon, B., Massart, D.L., 1994. Spectral mapping analysis: a method for the characterization of stationary phases. J. Chromatogr. Sci. 32, 488–498.

Hoffman, J.L., Bock, R.M., 1970. Interaction of cyclodextrins with nucleic acids. A study of secondary structure in three transfer ribonucleic acids. Biochemistry 9, 3542–3550.

Lewi, P.J., 1976. Spectral mapping, a technique for classifying biological activity profiles of chemical compounds. Arzneim. Forsch. 26, 1295–1300.

Lewi, P.J., 1989. Spectral map analysis. Factorial analysis of contrast, especially from log ratios. Chemom. Intell. Lab. Syst. 5, 105–116.

Mager, H., 1982. Moderne Regressionsanalyse, Salle, Sauerlander, Frankfurt am Main, pp. 135–167.

Nieto, M.I., Caamano, O., Fernandez, F., Gomez, M., Balzarini, J., De Clerq, E., 2002. Synthesis, antiviral and cytostatic activities of carbocyclic nucleosides incorporating a modified cyclopentane ring. IV. Adenosine and uridine analogues. Nucleosides Nucleotides Nucleic Acids 21, 243–255.

Oros, G., Cserháti, T., Forgács, E., 1997. Use of spectral mapping and stepwise regression analysis for the assessment of the relationship between chemical structure and biological activity of surfactants. Chemom. Intell. Lab. Syst. 39, 95–101.

Oros, G., Cserháti, T., Forgács, E., 1999. Inhibitory effect of nonionic surfactants on sunflower downy mildew. A quantitative structure–activity relationship study. Chemom. Intell. Lab. Syst. 47, 149–156.

Pichon, C., Feulon, I., Midoux, P., Mayer, R., Monsigny, M., Roche, A.-C., 1997. Cytosolic and nuclear delivery of oligonucleotides mediated by an amphiphilic anionic peptide. Antisense Nucleic Acid Drug Dev. 7, 335–343.

Sammon Jr., J.W., 1969. A nonlinear mapping for data structure analysis. IEEE Trans. Comput. C18, 401–407.

Szoka, F.C., Xu, Y., Zelphati, O., 1996. How are nucleic acid released in cells from cationic lipid–nucleic acid complexes? J. Liposome Res. 6, 567–587.

Takle, G.B., Thierry, S.M., Flynn, S.M., Peng, B., White, W., de Vonish, R.A., Galbraith, A.R., Goldberg, A.R., George, S.T., 1997. Delivery of oligoribonucleotides to human hepatoma cells using cationic lipid particles conjugated to ferric protoporphyrin IX (heme). Antisense Nucleic Acid Drug Dev. 7, 177–185.

Taylor, A.H., Pringle, J.H., Bell, S.C., Al-Azzawi, F., 2001. Specific inhibition of estrogen receptor alpha function by antisense

oligodeoxyribonucleotides. Antisense Nucleic Acid Drug Dev. 11, 219–231.

Vandeginste, B.G.M., Massart, D.L., Buydens, L.M.C., De Jong, S., Lewi, P.J., Smeyers-Verbeke, J., 1998. Handbook of Chemometrics and Qualimetrics. Part B. Data Handling in Science and Technology, vol. 20B. Elsevier, Amsterdam.

Zhao, Q.Y., Temsamani, J., Agrawal, S., 1997. Use of cyclodextrin and its derivatives as carriers for oligonucleotide delivery. Antisense Nucleic Acid Drug Dev. 7, 185–192.

Zobel, H.P., Kreuter, J., Werner, J., Noe, C.R., Kumel, G., Zimmer, A., 1997. Cationic polyhexylcyanoacrylate nanoparticles as carriers for antisense oligonucleotides. Antisense Nucleic Acid Drug Dev. 7, 483–493.