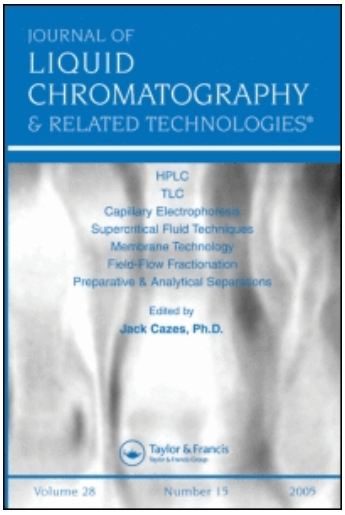


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# VANCOMYCIN AS A CHIRAL SELECTOR IN CENTRIFUGAL PARTITION CHROMATOGRAPHY

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## ABSTRACT

The use of vancomycin as a chiral selector was introduced by D. W. Armstrong. This macrocyclic antibiotic was used either free in solution for thin-layer chromatography (TLC) and capillary electrophoresis (CE) or covalently bonded to silica gel in the case of high performance liquid chromatography (HPLC). Its possible use in countercurrent chromatography (CCC) or centrifugal partition chromatography (CPC) is also of interest since these techniques have preparative capabilities which make them attractive for multigram scale production of enantiomers.

Two immiscible liquid phases coexist: an aqueous stationary phase containing vancomycin, and an organic mobile phase in which the racemic compound is more partitioned. This has a number of advantages over HPLC: lower stationary phase costs, ease of recovery of the antibiotic and exchange with a fresh batch, and, especially, improved resolution due to higher selectivity ($\alpha$).

Surprisingly, our experiments showed that chiral recognition in CCC & CPC is strongly solvent-sensitive. Acetonitrile, used by D. W. Armstrong at 10% or higher concentrations, was found to inhibit enantio-selectivity at levels as low as 5%. Inhibition was observed with most solvents, with the notable exception of toluene and related aromatics bearing no other heteroatom than halogens in their molecule. Exceptionally high selectivity factors ($\alpha > 30$) were observed for dansyl-norleucine in toluene-, ethylbenzene- and hexafluorobenzene- vancomycin 0.1 M (pH 4.7) systems. In a CPC run using toluene, injection of 50 mg dansyl-norleucine led to the isolation of 20.4 mg L isomer ($[\alpha]_D^{20} = -29.8°$) and 24.3 mg D isomer ($[\alpha]_D^{20} = +31.2°$). In HPLC using the commercial vancomycin Chiroptic V® column, both enantiomers were found to be free of contamination (ee # 100% ).



## INTRODUCTION

Macrocyclic antibiotics have recently been introduced by D. W. Armstrong et al. as powerful and "general" chiral selectors in liquid chromatography (LC),[1] thin-layer chromatography (TLC),[2] and capillary electrophoresis (CE)[3]. Native or derivatized vancomycin, rifamycin B, and thiostrepton have been used in both normal and reversed-phase mode in LC with the mobile phase composed of acetonitrile / 1% triethylammonium acetate buffer or 2-propanol / hexane.[1] A great variety of racemic compounds has been resolved on these chiral stationary phases, such as coumachlor and warfarin, devrinol, 5-methyl-5-phenyl-hydantoin, and various derivatized amino acids, with higher separation factors ($\alpha$) of 2.42 (coumachlor, pH 3.6). Vancomycin has been used as a chiral additive in a water / acetonitrile mixture to resolve 6-carbamyl-quinolyl N-protected amino acids (AQC), racemic drugs, and dansyl amino acids by TLC on diphenyl-type stationary phases.[2]

It would be interesting to determine whether macrocyclic antibiotics can also be used in countercurrent chromatography (CCC)[4-6] or in centrifugal partition chromatography (CPC),[7] since these techniques have preparative capabilities which make them attractive for multi-gram scale production of pure enantiomers.

CCC and CPC are liquid-liquid chromatographic techniques using a column consisting of long coiled tubing (CCC) or discrete channels linked in a cascade (CPC) and subjected to a variable (CCC) or constant (CPC) centrifugal field. The two phases (stationary and mobile) are immiscible liquids prepared by mixing two or more solvents or solutions. N-dodecanoyl-L-proline-3,5-dimethylanilide has recently been introduced in both elution[8-10] and displacement[9] modes, for resolution of (±)-dinitrobenzoyl amino acids.

Our preliminary studies of vancomycin as a chiral selector in CCC & CPC are reported here.

## EXPERIMENTAL

### Reagents

Vancomycin (V) (MW = 1449) purified by chromatography (HPLC purity ≈ 99%) was obtained from E. Lilly France S.A. (Saint-Cloud, France) in chlorhydrate form, in vials containing 500 mg (expressed in base). Dansyl-amino acids were obtained from Sigma (St Louis, MO, USA), and triethylamine, acetic acid, ammonia, hydrochloric acid, and all solvents came from Prolabo (Paris, France) and were of analytical grade. Water was deionized and filtered.

### HPLC, CCC & CPC

HPLC was used to control the partition experiments and the CCC & CPC runs. Two columns were used : a Cyclobond I (chiral selector = β-cyclodextrin) column (Astec, Whippany, NJ, USA) with a mobile phase composed of methanol / 0.1% triethylammonium acetate in water, 50 / 50 v/v, and a Chirobiotic V® (chiral selector = vancomycin) column (Astec), with a mobile phase of acetonitrile / 0.1% triethylammonium acetate, pH 4.3, in water, 15 / 85 v/v.

The pump was an Altex 110 (Touzart & Matignon, Vitry, France), and the detector a Spectro-monitor III (LDC, Pittsburgh, PA, USA) (UV detection at 254 nm). A Rheodyne model 7125 injector (Cotati, CA, USA) with a 20 µL sample loop was used.

CCC runs were performed on an HSCCC (P.C. Inc., Potomac, MD, USA) using the small coil of a triple coil [i.d. = 1.07 mm, total volume $V_c$ = 13 mL, β = 0.85 (ratio of the radius of rotation to the radius of revolution for this column)]. The rotational speed was 800 rpm. The pump was a P-500 model (Pharmacia, Uppsala, Sweden), and the injector a medium-pressure Rheodyne model 5011 injector with a 500 µL sample loop.

CPC runs were performed on an HPCPC (Sanki Engineering Limited, Kyoto, Japan) which was slightly modified: only one of the two packs of disks constituting the column was used, i.e. ≈ 1068 channels with a total volume, $V_c$ , of 90 mL. The rotational speed was 1500 rpm. Two model 510 pumps with an automatic gradient controller (Millipore, Waters Chromatography Div., Milford, MA, USA) were used with a Rheodyne model 7110 injector and a 5 mL sample loop. Mobile phases and exact procedures are described in the Results and Discussion Section.

## HPLC Procedure for Control of Partition Experiments and Chromatographic Runs

To estimate the partition of a racemic compound between an organic phase and an aqueous solution of vancomycin, an aliquot (100-200 µL) of the organic phase was evaporated and the residue dissolved in 200 - 500 µL of the mobile phase used in HPLC; 20 µL was injected. Enantiomers L and D (L and D were used instead of R and S since most of this work was done with amino acids) gave two peaks whose areas were $A_L$ and $A_D$. Assuming that the two enantiomers are initially found in equal quantities in the racemic compound, corresponding to a peak area $A^0$ for each enantiomer, we can calculate the distribution ratios, D (ratio of the total analytical concentration of a solute in one phase, regardless of its chemical form, to its total concentration in the other phase),[11] of the two enantiomers:

$$D_L = \frac{(\text{L in aqueous phase})}{(\text{L in organic phase})} = \frac{A^o - A_L}{A_L} \quad (1)$$

$$D_D = \frac{(\text{D in aqueous phase})}{(\text{D in organic phase})} = \frac{A^o - A_D}{A_D} \quad (2)$$

Assuming that $D_D > D_L$ (if not, the symbols must be inverted), the separation factor, α, is then:

$$\alpha = \frac{D_D}{D_L} = \frac{A_L}{A_D}\frac{A^o - A_D}{A^o - A_L} \quad (3)$$

$D_D > D_L$ means that $A_L > A_D$ (the L-enantiomer partitions more in the organic phase than the D-enantiomer), hence:

$$(A^o - A_D)/(A^o - A_L) > 1 \quad (4)$$

We can then define the enantioselectivity factor, α', as follows:

$$\alpha' = \frac{A_L}{A_D} \qquad (5)$$

Equations (3) to (5) indicate that α' is always smaller than α, which means that if a large value is found for α', that for α it is certain to be even larger. α' will be used instead of α in most cases, since its estimation requires only one HPLC run.

When an absolute estimation of $D_L$ and $D_D$ was necessary, the concentration of the two enantiomers in the aqueous phase was also estimated. An aliquot (200-400 µL) of the aqueous phase was acidified with HCl and extracted with an equivalent volume of methyl tert-butyl ether, which in most cases led to a total recovery of the enantiomer in the organic phase (as checked by a second extraction, resulting in a blank). Half the extract was evaporated, and the residue was dissolved in 200 - 500 µL of the mobile phase used in HPLC; 20 µL was injected. Enantiomers L and D gave two peaks whose areas were $A^W_L$ and $A^W_D$. Distribution ratios and the separation factor were then tabulated:

$$D_L = \frac{A^w_L}{A_L};\ D_D = \frac{A^w_D}{A_D};\ \alpha = \frac{D_D}{D_L} \text{ with } D_D > D_L$$

Most of our experiments were performed using dansyl-D,L-norleucine (DNS-Nle) as the racemic to be resolved.

## RESULTS AND DISCUSSION

Vancomycin (V) is soluble in water (> 140 mg/mL), somewhat soluble in methanol, and insoluble in higher alcohols and other less polar organic solvents. A typical biphasic system, suitable for chiral CCC & CPC, thus consists of a water-rich stationary phase containing V and a less polar mobile phase in which the racemic compounds preferentially stay, so that their partition ratio is in the range 0.5 to 1, favoring their rapid recovery.

### Primary Role of Solvents

Our first attempts with the heptane / ethyl acetate / methanol / water system failed because a gel occurred at high concentrations of V. Other systems such as non-polar solvent / methanol / water also failed because of gelling.

**Table 1**

**Solvent/Water Systems Tested to Study the Enantio-Selectivity of Vancomycin (V) Toward Dansyl-D,L-Norlecuine**

| Biphasic System* | V(mg/mL) | Enantio-Selectivity Factor, $\alpha'$ |
|---|---|---|
| BuOH | 37.5 | 1 |
| $CH_2Cl_2$/W | 37.5 | 1 |
| Hep/THF/W [19/45/36] | 120 | 1 |
| MtBE/0.6M NaCl | 120 | 1 |
| MtBE/Citrate buffer pH4 | 120 | 1 |
| MtBE/MeCN/0.6M NaCl [25/10/65] | 60 | 1 |
| MtBE/MeCN/Citrate buffer pH [25/10/65] | 120 | 1 |
| MtBE/W | 37.5 | 1 |
| EtOAc/W | 80 | 1 |
| MiBK/W | 80 | 1 |
| Cyclohexane/W | 60 | 1.01 |
| Tol/MeCN/Citrate buffer pH 4 [25/10/65] | 120 | 1.31 |
| Tol/W | 60 | 1.5 |
| Tol/MeCN/Citrate buffer pH 5 [30/5/65] | 120 | 1.74 |
| Tol/W | 120 | 15 |

* Abbreviations: BuOH: 1-butanol; W: water; Hep: heptane; THF: tetrahydrofuran; MtBE: methyl-*tert*-butyl ether; MeCN: acetonitrile; Tol: toluene; $\alpha$' is defined in experimental section.



Several solvents and solvent mixtures were then tested against water. The racemic compound chosen to test chiral recognition was dansyl-D,L-norleucine (abbreviated $R_{LD}$ for the racemic and $R_L$ and $R_D$ for the enantiomers). Results are summarized in Tables 1 and 2. Only aromatic solvents mixed with aqueous solutions of V allowed chiral recognition of $R_L$ from $R_D$. In the series of aromatic solvents, the enantio-selectivity factor was strongly dependent upon the nature and number of substituents. $-CH_3$ and -X increased $\alpha$', while $-CH_2OH$ or $-OCH_3$ decreased it. Hexafluorobenzene proved to be the best solvent for chiral recognition, but for practical reasons toluene was selected for further experiments.

## Effect of a Co-Solvent

As indicated in Table 1, adding a co-solvent such as acetonitrile in the biphasic toluene/aqueous solution of V system dramatically decreased chiral

**Table 2**

**Three Series of Aromatic Solvent/Water Tested to Study the Enantio-Selectivity of Vancomycin[a] (V) Toward Dansyl-D,L-Norleucine**

| Solvent | Enantio-Selectivity Factor, α' |
|---|---|
| **Series 1** | |
| Benzyl alcohol | 1.05 |
| Anisole | 1.06 |
| Furan | 1.09 |
| Thiophene | 1.11 |
| Benzotrichloride | 1.26 |
| Benzene | 1.27 |
| Nitrobenzene | 1.31 |
| Chlorobenzene | 1.57 |
| Toluene | 1.59 |
| 1,2-Dichloro-benzene | 1.87 |
| Xylene | 2.04 |
| Hexafluorobenzene | 3.63 |
| **Series 2** | |
| Toluene | 1.13 |
| Ethylbenzene | 1.71 |
| Hexafluorobenzene | 3.12 |
| **Series 3** | |
| Toluene | 1.58 |
| Divinylbenzene | 2.38 |
| Ethylbenzene | 3.34 |

[a] (V) = 60 mg/mL; three solutions of vancomycin were used for the three series, the pH of which was not controlled. They cannot be compared (see below the effect of pH).

recognition of enantiomers. Figure 1 shows the effect of adding acetonitrile in a biphasic toluene/water system containing 60 mg/mL of vancomycin. Five to 7 % acetonitrile in the mixture was sufficient to suppress chiral recognition of D- and L-DNS-Nle. This result is surprising since acetonitrile/water mixtures are used in HPLC with a vancomycin bonded to silica column,[1] and in TLC with vancomycin in the mobile phase.[2] It seems clear that the behavior of vancomycin in solution in liquid-liquid systems is totally different from that found when a solid phase is used.

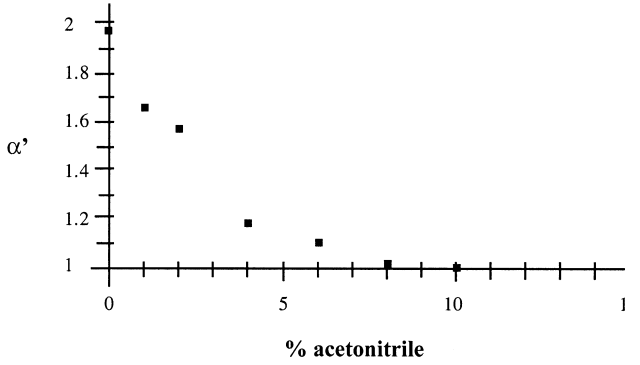


**Figure 1.** Influence of adding a co-solvent in the system toluene / aqueous solution of vancomycin (60 mg/mL) on the enantioselectivity factor α' for dansyl-D,L-norleucine.



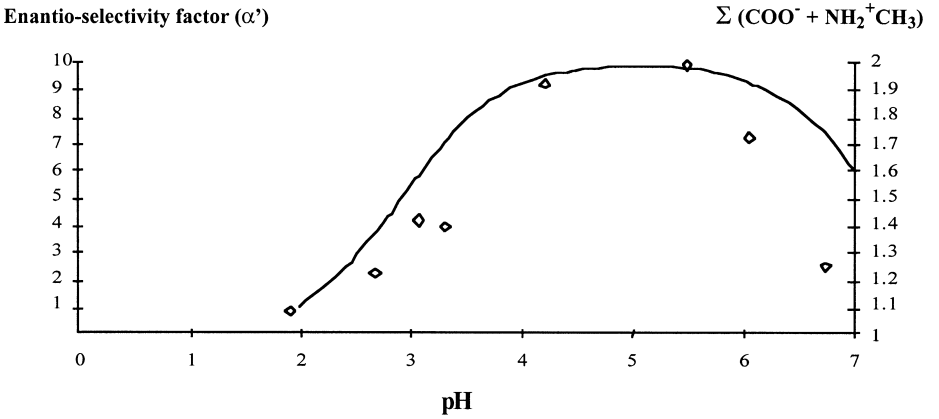


**Figure 2.** Influence of pH on the chiral recognition of dansyl-D,L-norleucine by the system toluene / aq. vancomycin (100 mg/mL). Experimental plots correspond to α' = f(pH); the line corresponds to the equation (for one molecule of vancomycin):

$$\sum(-COO^- + -NH_2^+CH_3) = \frac{10^{(pH-pK_{COOH})}}{1+10^{(pH-pK_{COOH})}} + \frac{10^{(pK_{NH_2CH_3}-pH)}}{1+10^{(pK_{NH_2CH_3}-pH)}}$$

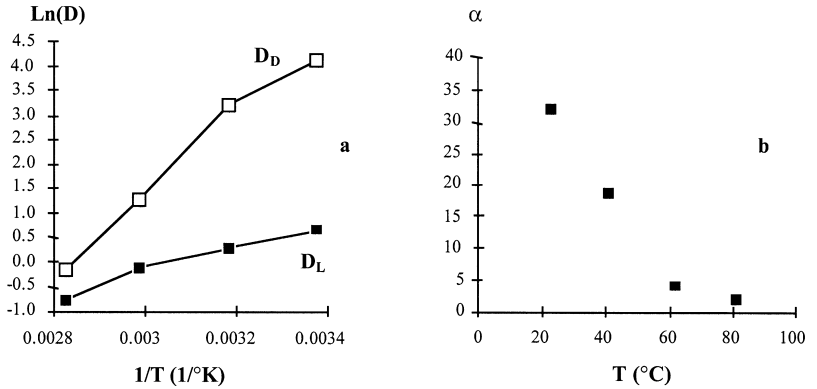


**Figure 3.** Dependence of the distribution ratio and of the selectivity factor with the temperature. System toluene / aq. vancomycin (140 mg/mL); the racemic compound is dansyl-D,L-norleucine.



## Effect of pH

Though the role of the organic solvent is critical, that of pH is also important, since chiral recognition was effective only in the pH 4 to 6 range (Figure 2). This may have been due to the ionizable nature of the racemic compound and to the ionizable functions of vancomycin with the following pKas:[12]

| | | |
|---|---|---|
| carboxylic acid: | | 2.9 |
| amine group of N-methyl leucine: | $-NH_2^+CH_3$ | 7.2 |
| amine of vancosamine: | $-NH_3^+$ | 8.6 |

As it was suspected that the first two groups play a role in chiral recognition, the abundance of the ionic form for these two groups was calculated in one molecule of vancomycin, as a function of pH, i.e.: f (pH) = $(-COO^-) + (-NH_2^+CH_3)$. The variation was compared with that of chiral recognition in Figure 2. The similitude between the experimental points and the calculated abundance suggests that the ionic interaction of these two sites with the racemic compound plays an important role in chiral recognition of ionizable molecules. The results are in agreement with conclusions found in two recent works.[13,14]

## Effect of Temperature

Figure 3 (a&b) shows the variation of the separation factor, α, with the temperature, and that of Ln(D) versus 1/T. Increasing temperature lowers the

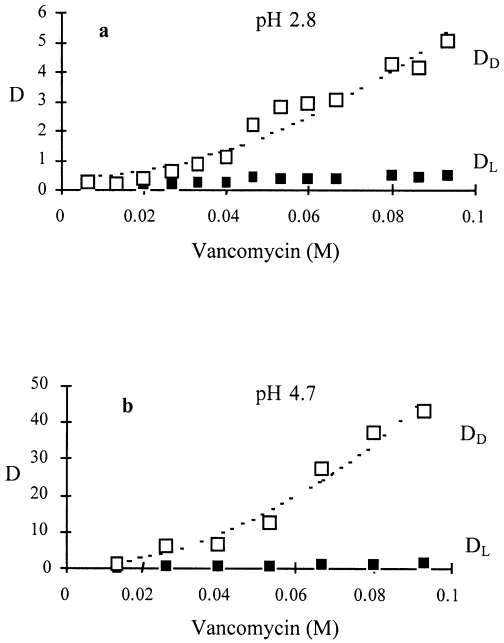


**Figure 4.** Variation of the distribution ratio of D- and L-dansyl-norleucine with the concentration of vancomycin in the system toluene / water. pH as indicated.



partition of the two enantiomers in the aqueous phase and decreases the separation factor. A regression analysis on plots Ln(D) = f(1/T) allowed an estimation of the standard energy of transfer, $\Delta G_{TR}$, of the two enantiomers in the partitioning between toluene and the aqueous solution of vancomycin [$\Delta G_{TR}$ = RT Ln(D)]. Calculated values were 20 kJ mole$^{-1}$ (std error: 3 kJ mole$^{-1}$) for DNS-L-Nle and 66 kJ mole$^{-1}$ (std error: 6 kJ mole$^{-1}$) for DNS-D-Nle. This large difference reflects the high selectivity of vancomycin for one enantiomer.

## Effect of Vancomycin Concentration

Several experiments were performed to estimate the influence of (V) on $D_L$ and $D_D$, (V) being varied from 10 to 140 mg/mL, i.e. 6.7 x $10^{-3}$ M to 9.3 x $10^{-2}$ M. In each case, the concentration of DNS-D,L-Nle was very small, i.e. around 0.3 mg/mL or ≈ 0.4 x $10^{-3}$ M for each enantiomer. This was negligible when compared to (V). Two experiments are reported here, performed at pH 2.8 (low recognition) and pH 4.7 (high recognition) respectively.

**Table 3**

**Linear Regression Analysis Performed on $D_L$ and $D_D = f(\bar{V}^2)$***

| DNS-D,L-Norleucine | | pH2.8 | pH 4.7 |
|---|---|---|---|
| L enantiomer | K (dimensionless) | 0.20 (0.02) | 0.4 (0.08) |
| | $KK^L_c$ ($M^{-2}$) | 39 (6) | 131 (19) |
| | R square | 0.80 | 0.9 |
| D enantiomer | K (dimensionless) | 0.4 (0.2) | 0.6 (2) |
| | $KK^D_c$ ($M^{-2}$) | 560 (40) | 5183 (415) |
| | R square | 0.94 | 0.96 |

*Data for $D = K(1 + K_c(\bar{V})^2)$, V in M.



Figure 4 (a and b) shows the results calculated with the HPLC controls of both organic and aqueous phases at equilibrium. The quadratic dependence of the distribution ratio versus the concentration of vancomycin suggests that a 1 : 2 complex was formed in the aqueous phase, corresponding to the following equilibria :

$$R_{LD} \overset{\leftarrow}{\rightarrow} \overline{R_{LD}} \qquad K = \frac{(\overline{R_{LD}})}{(R_{LD})} \quad \text{partition ratio} \qquad (6)$$

$$\overline{R_D} + 2\bar{V} \overset{\leftarrow}{\rightarrow} \overline{R_D V_2} \qquad K_c^D = \frac{(\overline{R_D V_2})}{(\overline{R_D})(\bar{V})^2} \quad \text{complex formation} \qquad (7)$$

$$\overline{R_L} + 2\bar{V} \overset{\leftarrow}{\rightarrow} \overline{R_L V_2} \qquad K_c^L = \frac{(\overline{R_L V_2})}{(\overline{R_L})(\bar{V})^2} \quad \text{in the aqueous phase} \qquad (8)$$

Hence the distribution ratio of the D isomer is:

$$D_D = \frac{(\overline{R_D}) + (\overline{R_D V_2})}{(R_D)} = K(1 + K_c^D(\bar{V})^2) \qquad (9)$$

$$D_L = \frac{(\overline{R_L}) + (\overline{R_L V_2})}{(R_L)} = K(1 + K_c^L(\bar{V})^2) \qquad (10)$$

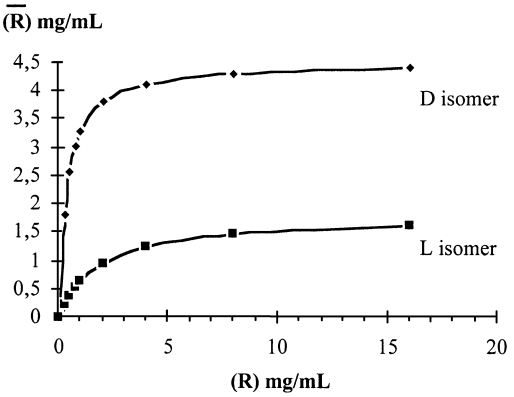


**Figure 5.** Langmuirian isotherms of D and L dansyl-norleucine in the system toluene/aq. vancomycin (140 mg/mL). ( ) in the aqueous phase; ( R ) in the toluene phase.



Linear regression analysis performed on $D = f[(\overline{V})^2]$ gave the results summarized in Table 3. K should have been the same for the L and D enantiomers at a given pH, and the disparity found probably reflects too schematic an approach (other complexes may also have been formed). However these results provide an estimation of the complex formation constant between vancomycin and DNS-D,L-Nle. At pH 4.7, $K_C^L \approx 327\ M^{-2}$ was found for the L enantiomer, and $K_C^D \approx 8.6\ 10^3\ M^{-2}$ for the D enantiomer. This means that the $R_D V_2$ complex is stable at pH 4.7 in the toluene/water system.

As noted above, this stability is solvent-dependent, since acetonitrile (10 %, v/v) added in the toluene / water biphasic system destroys the complex. This makes it easy to recover the pure enantiomer in the organic phase, and the pure vancomycin in the aqueous phase, after resolution of both enantiomers by CCC or CPC.

## Effect of the Concentration of the Racemic Compound

The preparative capabilities of CCC and CPC require knowledge of the relationship between the equilibrium concentrations of the solute in the mobile and stationary phases over a sufficiently wide range, i.e. the pertinent adsorption isotherms. It is often claimed that CCC and CPC does not show non-linear chromatographic patterns because the volume ratio of the stationary to the mobile phase is very high compared to that found in liquid-solid column chromatography.[7]

**Table 4**

**Parameters of the Langmuir Isotherms of D- and L-Dansyl-Norleucine***

| | a (dimensionless) | b (mL/mg) | B $Mole_{vancomycin}$/Mole | R Square |
|---|---|---|---|---|
| D isomer | 12.05 (3.5) | 2.67 (0.05) | 90.84 (2.0) | 0.998 |
| L isomer | 0.95 (0.2) | 0.52 (0.03) | 17.62 (1.1) | 0.980 |

* Equations 11 and 12.



However when a complex formation in the stationary phase is the main factor governing the retardation of an enantiomer, a Langmuirian-shaped isotherm can be expected. Figure 5 shows the experimental plots determined by the shake-flask method in the biphasic system [toluene / (V) = 140 mg/mL in water, pH 4.8, and T = 23°C], and the lines correspond to the Langmuir model:

$$(\overline{R_D})_{total} = \frac{a(R_D)}{1 + b(R_D)} \quad (11)$$

$$(\overline{R_L})_{total} = \frac{a(R_L)}{1 + b(R_L)} \quad (12)$$

with a and b being determined by linear regression analysis (Table 4).

The saturation concentrations $(\overline{R_D})^0$ and $(\overline{R_L})^0$, as given by a/b, are 4.5 mg/mL for $(\overline{R_D})^0$ and 1.8 mg/mL for $(\overline{R_L})^0$ (with 140 mg/mL of vancomycin). When expressed in $mole_{enantiomer}$ / $mole_{vancomycin}$, this corresponds to 0.13 mole / $mole_{vancomycin}$ for the D enantiomer.

In fact, at the concentration of 140 mg/mL used, it would be naive to expect a stoechiometric relationship. The V molecules are more likely linked via intermolecular hydrogen bonding interactions, leading to the formation of a liquid crystal phase.[15] The glycopeptide family of antibiotics is known to form dimers in solution,[16-20] which have been observed and studied at concentrations of up to 2 mg/mL. The aggregation of vancomycin in aqueous solution, as previously deduced from circular dichroism spectra, was found to be total at a concentration of about 10 mg/mL.[21] At concentrations above 100 mg/mL, formation of a supramolecular structure is likely, which would fit the following observations :

* V molecules are linked through hydrogen bonding, which is sensitive to organic solvents.

* The behavior of aromatic solvents may be due to the involvement of the phenolic groups in the stacking phenomenon or in the complexation involving π-π interactions.

Commercial Chirobiotic V® (vancomycin) and T® (teicoplanin) phases formed by covalent linking of the antibiotics to 5 μm silica[1] probably works well with acetonitrile/water and methanol/water eluents because of the establishment of a pure water bilayer at the surface of the hydrophilic silica. Chiral recognition probably occurs in this bilayer. Coverage of the silica surface is very important, and efficient phases need the highest antibiotic/silica ratio possible.



**Other Dansyl Amino Acids**

A rapid survey of the chiral recognition of 10 dansyl amino acids by vancomycin was performed using the following standard conditions: toluene / (V) = 80 mg/mL in water, pH 4.8, at 20°C. Chiral recognition occurred only for dansyl amino acids containing a hydrophobic side chain (Leu, Val, Nval, Phe, Met, Gaba), whereas dansyl amino acids containing a hydrophilic side chain (Thr, Ser, Glu, Asp) partitioned mainly in the polar aqueous phase, which excludes any measurable chiral recognition with the biphasic system. Other racemics are being investigated in our laboratory, and the results will be reported later.

**Application to CCC & CPC**

Two series of chromatographic runs have been performed, one using an HSCCC apparatus with a small 13 mL column, and other using an HPCPC apparatus with a medium 90 mL column (see Experimental). In both cases, the biphasic system was a mixture of toluene and an aqueous solution of 140 mg/mL vancomycin, adjusted to pH 4.7 by addition of ammonia.

To prepare minimum quantities of vancomycin solutions, the HPCPC instrument was first filled with toluene-saturated water. About half of the column volume of the vancomycin solution was then introduced at the ascending mode inlet of the HPCPC. The column was then rotated at the desired speed, and the water-saturated toluene phase was allowed to percolate at the chosen flow rate (5 mL/min), and in the right way, i.e. ascending mode. The small column of the HSCCC was filled with the vancomycin solution, then rotated at the desired rotational speed and percolated with the mobile phase until equilibrium was reached. The DNS-D,L-Nle sample was then injected, and

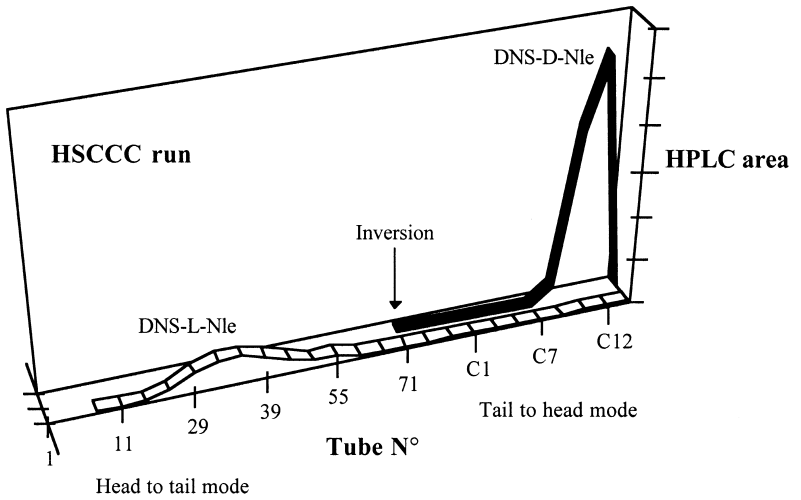


**Figure 6.** Dual-mode elution of L- and D-dansyl-norleucine using a HSCCC instrument. $V_c$ = 13 mL; $V_{mobile\ phase}$ = 6.2 mL; Flow rate = 0.6 mL/min. Rotational speed = 800 rpm; (vancomycin) = 140 mg/mL in water; sample = 5 mg in 500 µL. Collected fractions: 10 mL in tail to head mode; 4 mL in head to tail mode.



collected fractions were controlled with a trivial UV lamp used for TLC monitoring (model UVSL-25 Mineralight lamp, multi-band UV 254-366 nm, UV Products, Inc., San Gabriel, CA, USA). The chiral purity of the fluorescent fractions was checked by HPLC, and the CCC or CPC chromatograms were reconstructed from these measurements.

Figures 6 and 7 show two typical chromatograms. Dual-mode elution was performed since the D enantiomer was strongly retained in the vancomycin aqueous solution. After the L enantiomer was collected in the tail-to-head mode for the HSCCC, or in the ascending mode for the HPCPC, the valve mode was switched and toluene-saturated water was pumped into the column, which pushed out the vancomycin aqueous solution containing the D enantiomer. The D enantiomer was then extracted with methyl tert-butyl ether or toluene / acetonitrile and controlled by HPLC. Yields and optical rotation of enantiomers were checked for the run corresponding to Figure 7. For this run, 50 mg of DNS-D,L-Nle were injected in 5 mL of the upper phase. Fractions 4 to 18 (150 mL) gave 20.4 mg of DNS-L-Nle, with $[\alpha]_D^{20°}$ = - 29.84° ± 0.7 (c = 0.5, methanol), and fractions D23 to D33 (44 mL) gave 24.3 mg of DNS-D-Nle, with $[\alpha]_D^{20°}$ = + 31.2° ± 0.8 (c = 0.5, methanol). Yields were 82% for the L isomer and 97% for the D isomer.

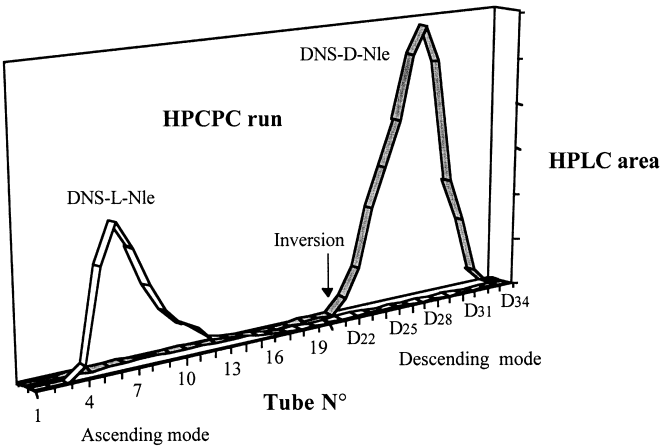


**Figure 7.** Dual-mode elution of L- and D-dansyl-norleucine using a modified HPCPC instrument. $V_c$ = 90 mL; $V_{mobile\ phase}$ = 25 mL; Flow rate = 5 mL/min. Rotational speed = 1500 rpm; (vancomycin) = 140 mg/mL in water; sample = 50 mg in 5 mL. Collected fractions : 10 mL in ascending mode; 4 mL in descending mode.



## CONCLUSION

Two main conclusions can be drawn from our experiments:

1. The experimental conditions in which vancomycin in solution will recognize an enantiomer are extremely narrow:

* an aromatic solvent is needed to make a biphasic system with water containing vancomycin;

* addition of a non-aromatic solvent destroys chiral recognition;

* the pH range is narrow;

* the concentration of vancomycin must be high to obtain high selectivity.

2. Are the same conditions needed for other classes of racemics? A major drawback preventing vancomycin from being a powerful chiral selector for preparative CCC and CPC is its molecular weight (MW ≈ 1400). Since two molecules of vancomycin are needed to complex one molecule of enantiomer, and since the Langmuirian shape of the isotherm suggests that it could be a supra-molecular organization involving 4 to 6 additional molecules of vancomycin, injected quan-

tities of racemic will remain small, even if selectivity is high. In our opinion an ideal chiral selector for CCC and CPC must have low molecular weight in order to achieve a high molarity in a solvent by dissolving a reasonable mass of it. Since chiral chromatography involves the formation of a complex between an enantiomer and the chiral selector, it is probable that Langmuirian-shaped isotherms will always be encountered. Thus, one way of extending the linear zone of these isotherms is to use higher molarities of chiral selector. A favorable alternative would be this chiral selector to be one of the solvents making the biphasic system.

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