Effect of achiral support on the resolution of tetramisole by supercritical fluid extraction

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Abstract—The enantiomers of tetramisole were produced by partial diastereomeric salt formation with O,O'-dibenzoyl-$(2R,3R)$-tartaric acid monohydrate and subsequent supercritical fluid extraction (SFE) of the unreacted enantiomers in the presence of an achiral support. The effect of the activated carbon and Perfil 100™ on the efficiency of chiral separation were studied. The kinetics of the process was found to be an important factor affecting enantioselectivity. When the parameters were properly set, much better resolution efficiency (F) and higher enantiomeric purity were achieved than in the equilibrium. The presence of Perfil 100™ and activated carbon caused an increase as high as 53 and 84% in F, respectively, compared to that achieved without using any support. Thus during the development of a resolution procedure, beside the proper resolution agent, optimised molar ratio and other parameters, the selection of achiral additive(s) may be also an important point. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

According to the US Food and Drug Administration's policy statement, marketing of new chiral pharmaceuticals has been allowed only as pure enantiomers from the beginning of 1992, except when the therapeutic properties of the racemic mixture are better.[1] In many cases these enantiopure drugs are produced by asymmetric synthesis followed by chiral resolution.

In recent years growing interest has been shown in new and environmentally friendly resolution processes.[2] Beside membrane separation, procedures using supercritical fluids have also become promising on industrial scale.[3,4] Our group developed a novel technique based on partial diastereomeric salt formation followed by supercritical fluid extraction (SFE) of the free enantiomers.[3] The method was successfully applied to the resolution of racemic organic acids and bases. Meanwhile, Bauza et al. also proved the feasibility of SFE of enantiomers for analytical purposes.[5,6]

The development of a widely applicable method requires a deep understanding of the influencing factors. The effect of the molar ratio, extraction pressure and temperature on the resolution of different organic acids[3] and bases has been reported recently.[7] Herein the effect of the achiral support is examined.

2. Materials and methods

Racemic tetramisole (6-phenyl-2,3,5,6-tetrahydro-imi-dazo-[2,1b]-thiazole) was prepared in our laboratory and checked analytically by HPLC prior use. It was found to be of 99.9% purity. The resolving agent (O,O'-dibenzoyl-$(2R,3R)$-tartaric acid = DBTA) and all other reagents and solvents were purchased from Reanal Ltd., Budapest.

Perfil 100™ (expanded and milled perlite for use as a filtering-aid) was obtained from Baumit Co. (Budapest). The activated carbon applied was of medical grade, commercially available in pharmacies. The specific surface area ($S_{a,BET}$) and the total pore volume values are listed in Table 1.

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Table 1. Specification of the supports

Support	$S_{a,BET}$[a] (m²/g)	Total pore volume[a] (cm³/g)	$S_{a,aval}$[b] (m²/g)
Active carbon	1.72×10^3	1.02	82.9
Perfil 100™	2.89	5.02×10^{-3}	2.89

[a] Determined from low temperature N_2-adsorption isotherm (AUTOSORB, QUANTACHROME, USA).
[b] It was supposed that pores below a diameter of 10 Å are not available for the molecules studied. In the case of the highly porous activated carbon sample, the $S_{a,aval}$ value (available specific surface area) was derived from the t-plot of the nitrogen adsorption data.[8]

The CO_2 used in this study was 99.5% (w/w) pure and supplied by Messer Griesheim Hungaria Ltd. (Budapest).

The resolution process is shown in Scheme 1. A description of the extractor unit is given in a previous paper.[9] In the sample preparation step the resolving agent and racemic tetramisole, in 0.25:1 molar ratio, were dissolved in an appropriate solvent, in this case methanol, and then the support was added to the clear solution. After about 5 min the solvent was evaporated in vacuum at 40°C and the mixture was dried at 40°C for 24 h. The extractor vessel was filled with this dry material and then the SFE was performed at 160 bar and 39°C.
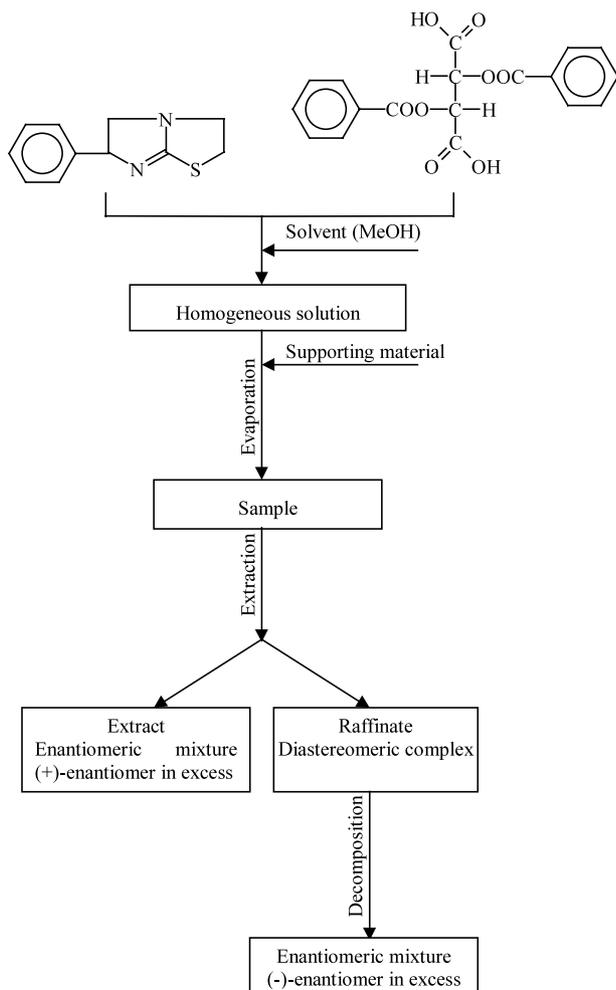
Scheme 1. Resolution by supercritical fluid extraction.

Optical rotation (α) measurements were carried out on a Perkin–Elmer 241 polarimeter, and the enantiomeric excess values were determined from α values according to prior calibration.

Adsorption isotherms were derived from the initial and equilibrium concentration of the liquid phase measured by a UV–Vis spectrophotometer (Uvikon Kontron, Zürich) at $\lambda = 224$ nm.

TG and DSC measurements were carried out on TA Instruments 2050 TGA V5.3C and TA Instruments 2920 MDSC V2.4F, respectively. Argon atmosphere was used in a 10 L/h flow rate. The mass of samples was 4–5 mg.

3. Results

3.1. Extraction curve

A typical extraction curve is shown in Fig. 1. The extraction yields (Y_e = mass of extract/mass of tetramisole in the sample) are plotted versus the specific solvent mass (w = mass of CO_2 passed through the extractor/mass of tetramisole in the prepared sample). The $Y_e = a(1 - e^{-kw})$ regression function suggested by Brunner[10] fits very well to the measured data. The quality of the support had no significant effect on the estimated parameters (a, k).

3.2. Adsorption of tetramisole and DBTA on Perfil 100™ and activated carbon

The adsorption isotherms were derived as $n_a = V(c_0 - c)/m$, where c_0 and c are the initial and equilibrium concentration, V is the volume of the solution and m is the mass of the support. The n_a versus c function was fitted according to the Langmuir model.[11] The deduced parameters, the monolayer capacity (n_m) and the adsorption constant (K) are listed in Table 2. The K values are characteristic to the adsorption 'affinity'. The low K values mean a weak adsorption. The strongest among them is the tetramisole/activated carbon interaction. Comparing the $m_{rel,m}$ and $m_{rel,res}$ values, it is obvious that in the experiments we were far above the monolayer capacity of the support, as the $m_{rel,m}$ is at least one order of magnitude smaller than $m_{rel,res}$.

Ye=(0.323)(1-exp(-0.0267w))
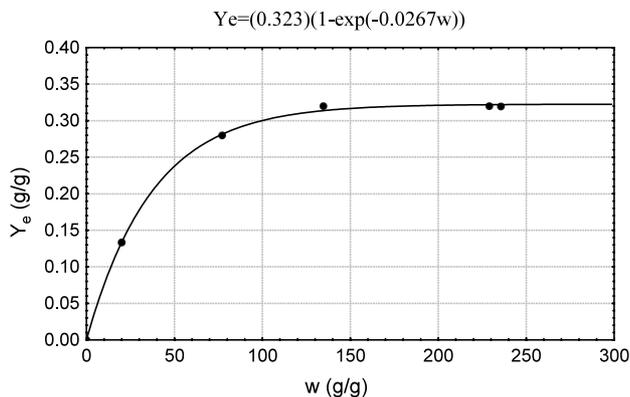
Figure 1. A typical extraction curve; $R^2 = 0.999$.

3.3. Thermal and X-ray diffraction analysis of the equilibrium samples

Samples for thermal analysis were prepared by slow evaporation of the solvent at 40°C. We supposed that during the slow evaporation an equilibrium state of the various phases was established. The DSC curves of tetramisole samples are presented in Fig. 2. The results are summarised in Table 3. The presence of the supports does not affect the melting point of the crystalline tetramisole, but the shape and the integral of the peaks are influenced by both the Perfil 100™ and the activated carbon. The deviation of the reported Q_m/Q_{cal} values from **1** suggests, that a part of the tetramisole is present in non-crystalline form. The decrease of crystalline tetramisole in equilibrium samples was proved by the X-ray diffraction measurements. [HCG-4 Carl Zeiss (Jena)]. It could be concluded, that either the presence of the support, or the long contact time due to the slow evaporation, suppress crystal formation.

A significant effect was also observed in the case of the DBTA/support systems. No peak was detected in the temperature range studied in the case of DBTA on activated carbon. When Perfil 100™ was added, an endothermic peak was detected in the range of the decomposition temperature of the DBTA.

Table 2. Parameters fitted from the linear Langmuir plot[11]

Support	Adsorbate	n_m (mmol/g)	K (dm^3/mg)	R^2	$m_{rel,m}$[a] (mg/m^2)	$m_{rel,res}$[b] (mg/m^2)
Activated carbon	Tetramisole	0.0090	1.247	0.997	0.022	10–350
	DBTA	0.3129	0.2742	0.998	0.589	4.6–160
Perfil 100™	Tetramisole	0.0026	0.0698	0.994	0.184	100–1100
	DBTA	Adsorption was below the detection limit				46–760

[a] $m_{rel,m}$ is the mass of tetramisole or DBTA/surface of the support at monolayer adsorption.
[b] $m_{rel,res}$ is the mass of tetramisole or DBTA/surface of the support at resolution experiments.
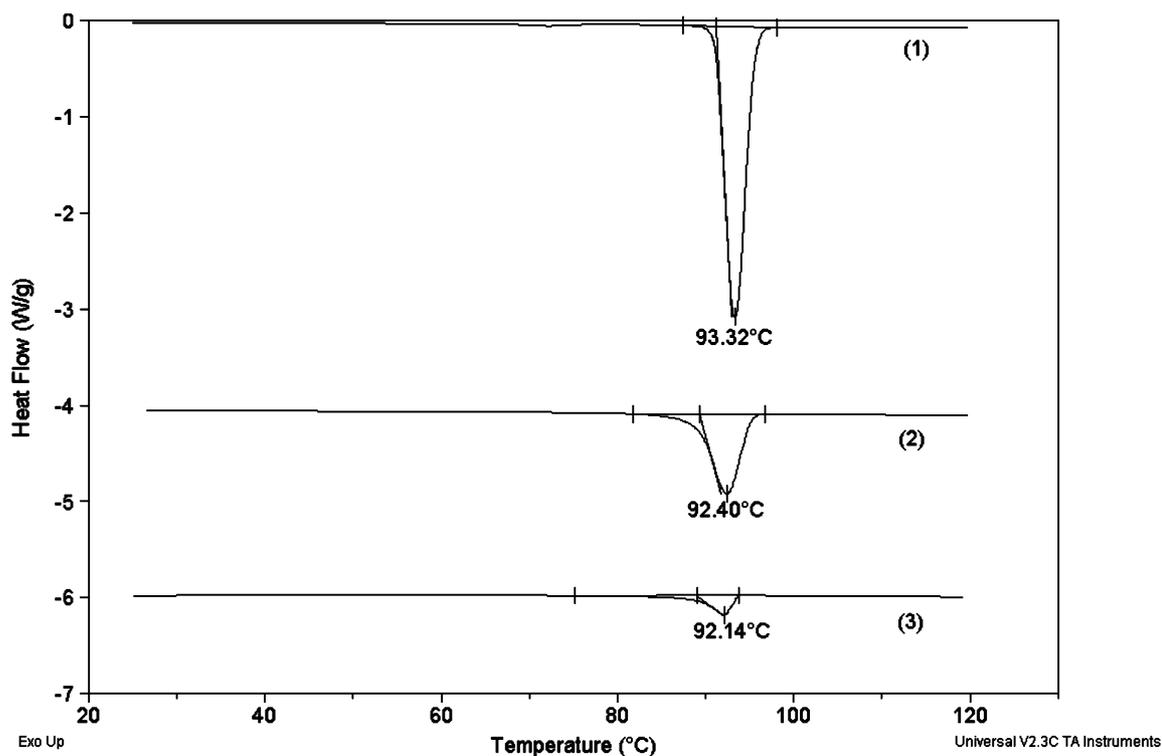
Figure 2. DSC curves of tetramisole samples; (1) pure tetramisole; (2) tetramisole on Perfil 100™; $m_{rel} = 0.373$, (3) tetramisole on activated carbon, $m_{rel} = 0.012$.

Table 3. DSC measurement results of tetramisole equilibrium samples

Support	m_{rel} (g/m^2)	Q_{cal}[a] (J/g sample)	Q_m[b] (J/g sample)	Q_m/Q_{cal}[c]
None (pure)		101.2	101.2	1
Activated carbon	0.012	50.73	8.31	0.164
Perfil 100™	0.373	48.67	41.36	0.850

[a] $Q_{cal} = Q_{pure}/(1 + m_{rel}S_{a,aval})$ is the calculated melting enthalpy from the amount of tetramisole. Q_{pure} is the melting enthalpy of tetramisole.
[b] Q_m is the measured melting enthalpy.
[c] By the ratio Q_m/Q_{cal} the crystalline ratio of tetramisole (mass of crystalline tetramisole versus mass of tetramisole in the sample) was estimated.

3.4. Effect of the support on chiral recognition

To evaluate the resolution efficiency, a merged F parameter was used ($F = |Y_e ee_e| + |Y_r ee_r|$, where Y_r is the yield of tetramisole in raffinate, ee_e and ee_r are enantiomeric excess values of extract and raffinate, respectively). F may vary between 0 and 1. According to the mass balance the terms $|Y_e ee_e|$ and $|Y_r ee_r|$ should be equal. This new parameter provides a better estimation of the resolution efficiency of the extract and raffinate, and the degree of freedom becomes higher at statistical evaluation.

Experimental results are shown in Figs. 3 and 4. The F/F_0 term shows the relative effect of the support on resolution efficiency. F_0 is the resolution efficiency obtained without using any support ($F_0 = 0.341 \pm 0.0236$).

An increase in the m_{rel} values leads to a continuous increase of F/F_0 up to the limits 1.53 and 1.84 using activated carbon and Perfil 100™, respectively.

4. Discussion

After observing the interesting effects of the support, our first aim was to establish whether these were caused by the change in diastereomeric equilibrium, or resulted from kinetic control of the resolution. To test this, a sample was stirred with activated carbon ($m_{rel} = 0.094$ g/m^2) for 1 h prior to evaporation of the solvent (to reach the equilibrium). Much lower selectivity was achieved ($F/F_0 = 0.998$), than calculated from the regression function ($F/F_0 = 1.755$). Surprisingly the measured F was almost the same as obtained without using any support. These results suggest that the higher resolution efficiencies at higher m_{rel} values are caused by non-equilibrium, kinetic process and the decrease of the crystalline phase at quasi-equilibrium state (see part 3.3) suppress the chiral recognition as well.

Almost the same results were achieved, when only the solution of tetramisole or only the solution of DBTA were stirred for an hour with activated carbon and the solution of the other compound was added to the mixture just before the evaporation step (see Table 4). Although tetramisole showed higher adsorption 'affinity' than DBTA (see Section 3.2), according to

these results it is not likely, that the adsorption of tetramisole results in a change of F/F_0.
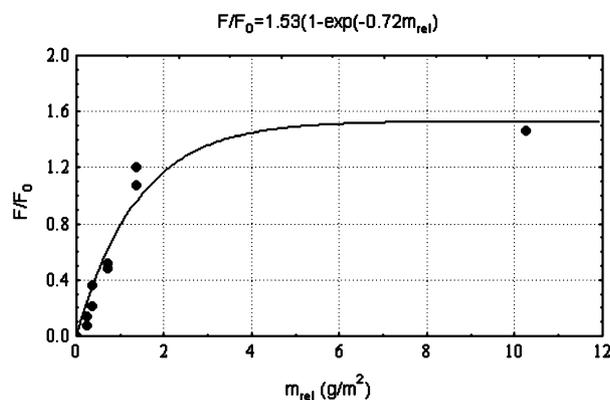
Figure 3. Effect of the specific mass of tetramisole on perfil (m_{rel}) on resolution.
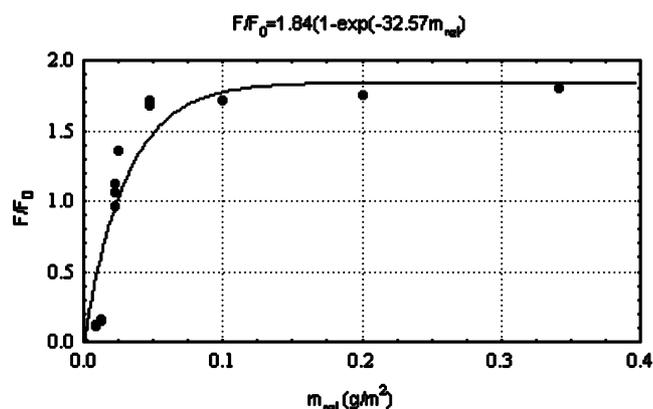
Figure 4. Effect of the specific mass of tetramisole on activated carbon (m_{rel}) on resolution.

Table 4. Effect of sample preparation on the resolution efficiency. The solution of the given compound was stirred 1 h with the activated carbon then the solution of the other compound was added only 5 min before the evaporation

Stirred compound	m_{rel} (g/m^2)	F/F_0 measured	F/F_0 calculated[a]
Tetramisole	0.095	0.902	1.777
DBTA	0.094	0.909	1.771

[a] From the regression function in Fig. 4.

According to the adsorption data (K values) none of the components studied show a strong adsorption interaction. Thus, among the components present in the prepared sample, the adsorption of a diastereomeric complex may be the strongest, due to its higher molecular mass and lower solubility. Since the solubility of (S)-(−)-tetramisole–DBTA diastereomer in methanol is much lower than that of (R)-(+)-tetramisole–DBTA diastereomer, the (S)-(−)-tetramisole-containing diastereomer has the highest probability to adsorb. All these factors suggest, that the higher chiral separation at higher m_{rel} values is due to simple competition of the components for adsorption sites. The adsorbed molecule may induce its own crystallisation, and the (S)-(−)-tetramisole-DBTA diastereomer is the most likely to start this. When there is a huge amount of bonding sites, all possible molecules in the solution adsorb and start to crystallise, lowering the chiral differentiation toward zero.

5. Conclusions

This resolution procedure by partial diastereomeric salt formation followed by supercritical fluid extraction of the unreacted enantiomers is a form of kinetic resolution. When process parameters are properly set, much better resolution efficiency and higher enantiomeric purity can be achieved than at the equilibrium.

In the development of a resolution procedure, besides choosing an appropriate resolving agent, molar ratio and other parameters, the selection of achiral additive(s) is also an important aspect.

6. Experimental

Racemic tetramisole (1.50 g, 7.40 mmol) and O,O'-dibenzoyl-(2R,3R)-tartaric acid monohydrate (0.69 g, 1.85 mmol) were dissolved in methanol (20 mL). Active carbon (0.05g) was added to the clear solution and the solvent was evaporated at 40°C under reduced pressure (water vacuum). The residue was extracted with supercritical carbon dioxide (160 bar, 39°C, 900 g CO_2). The (R)-(+)-enantiomer (0.64 g) ($[\alpha]_D^{20} = +72.6$ (c 5, MeOH)) was recovered from the extract. The raffinate was suspended in water (20 mL) and NH_4OH was added (25 w/w%) to pH 9.5. The reaction mixture was stirred for 30 min at 10°C and the (S)-(−)-enantiomer. (0.66 g) was isolated by filtration ($[\alpha]_D^{20} = -78.8$ (c 5, MeOH)).

A description of the enantiomeric enrichment by partial salt formation with HCl and subsequent SFE was given in a previous paper.[7] Scheme 2 shows the whole resolution process with results of the given experimental. The higher enantiomeric excess achieved in the first step, with addition of activated carbon, results in higher total yield values (Y_{total} = mass of pure enantiomer/mass of racemic tetramisole in the initial sample), see Table 5.

Scheme 2. The whole resolution process of tetramisole using activated carbon at $m_{rel} = 0.347$ and 5 min sample preparation time.

Table 5. Comparison of yields of whole resolution procedures

Method	First step, mixture with (R)-(+) in excess		First step, mixture with (S)-(−) in excess		Second step Y_{total}	
	Y	ee	Y	ee	(R)-(+)	(S)-(−)
Optimised SFE[8]	0.404	0.600	0.421	0.540	0.189	0.211
SFE, without support	0.391	0.346	0.415	0.512	0.158	0.188
SFE, perfil	0.472	0.582	0.428	0.547	0.224	0.199
SFE, activated carbon[a]	0.427	0.680	0.440	0.737	0.221	0.269

[a] See Scheme 2.

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