Recognition of Barbiturates in Molecularly Imprinted Copolymers using Multiple Hydrogen Bonding

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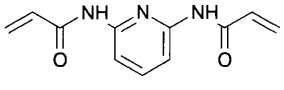
Barbiturate-selective copolymers were prepared using the molecular imprinting technique in combination with a functional monomer, 2,6-bis(acrylamido)pyridine, capable of multiple hydrogen bond formation with the barbiturate templates.

Molecular imprinting is a template polymerization technique involving covalent and/or noncovalent bond formation between templates and monomers having functional groups for interaction with the templates (functional monomers) during polymerization. Because of the ease of the procedure, many compounds have been studied using this technique, including sugars, amino acid derivatives, drugs, agrochemicals *etc.*[1]

In molecular imprinting systems using noncovalent bond formation, multiple interactions between templates and functional monomers are important for not only recognizing the templates but also producing ligand-selective recognition sites in the imprinting polymers, because of the stabilization of template–functional monomer complexes during polymerization. To date, however, only single functional group bearing monomers such as methacrylic acid have been used, even though, on entropic grounds,[2] suitable simultaneous multiple interactions should prove superior. Itaconic acid has previously been reported[3] for use in an imprinting application, though the lack of good monomer–template complementarity offered little advantage.

In this study, 2,6-bis(acrylamido)pyridine **1**, capable of formation of multiple hydrogen bonding with barbiturate template structures, was used for the first time in molecular imprinting applications, and its effectiveness was demonstrated by the preparation of molecularly imprinted polymers for barbiturates which are hypnotic agents used in therapy for insomnia.

The functional monomer **1** was prepared in the manner reported previously.[4] The formation of hydrogen bonding between **1** and cyclobarbital **2** in the pre-polymerization stage was verified by ^{1}H NMR studies. Addition of cyclobarbital to the functional monomer dissolved in $CDCl_3$ led to downfield shifts caused by hydrogen bond formation of the diaminopyridine unit of the functional monomer with cyclobarbital.



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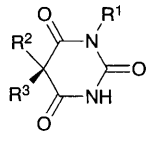
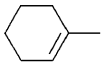
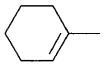
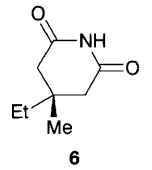


	R^1	R^2	R^3
2	H		Et
3	H	$CH_2{=}CHCH_2$	$CH_2{=}CHCH_2$
4	H	Et	$Me_2CH[CH_2]_2$
5	Me		Me



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Cyclobarbital-imprinted copolymers were prepared as follows: compounds **1** (1 mmol), **2** (0.5 mmol) and ethylene glycol dimethacrylate (20 mmol) were dissolved in chloroform, and the mixture was purged with nitrogen gas. The polymerization was initiated by the addition of 2,2-azo(2,4-dimethylvaleronitrile) and the reaction mixture was incubated for 24 h at 45 °C, and then for 3 h at 90 °C. The polymer obtained was ground and sieved to yield polymer particles in the size range 26–63 μm. The sieved polymer was packed into stainless steel columns (100 mm × 4.6 mm I.D.) and was washed exhaustively with methanol and chloroform to remove the template.

The selectivity of the cyclobarbital-imprinted polymer was assessed by comparison of the chromatographic behaviour of the template **2**, allobarbital **3**, amobarbital **4**, hexobarbital **5** and the structurally related 3-ethyl-3-methylglutarimide **6** on the cyclobarbital-imprinted column (Table 1). The relative retentions of these probes using chloroform as eluent were compared with their retention behaviour on a column packed with nonimprinted polymer which was prepared by the same procedure but without the template.

The imprinted polymer retained barbiturates, while none of the compounds tested was adsorbed on the nonimprinted polymer. As both polymers have the same monomer composition, differences in the retention behaviour can be interpreted in terms of the imprinting effects. The cyclobarbital-imprinted polymer showed a distinct preference for the template structure **2**. Allobarbital was also strongly retained, however amobarbital showed only one half of the k' value of cyclobarbital and low retention was observed for hexobarbital and 3-ethyl-3-methylglutarimide.

Comparison of the retention behaviour and ligand structural differences showed the nitrogen atoms of the malonylurea structure to be significant for binding. Hexobarbital with an *N*-methyl group on the malonylurea structure was not retained, and **6**, possessing a single *N*-methyl group, showed a lower k' value than other barbiturates. These results suggest that the formation of hydrogen bonding is inhibited by the *N*-methyl group, and a half-binding interaction is not sufficient for high affinity binding. The binding seems to be little affected by various

Table 1 Retention behaviour of compounds tested in the cyclobarbital-imprinted polymer

		Capacity factor[a]				
Polymer	Eluent	**2**	**3**	**4**	**5**	**6**
Imprinted	$CHCl_3$	12.4	11.9	6.72	0.39	1.82
	MeOH	1.38	2.04	0.46	0.33	0.47
Nonimprinted	$CHCl_3$	0.34	0.19	0.13	0.61	0.23
	MeOH	0.21	0.07	0.01	0.58	0.21

[a] The polymers were packed in stainless steel columns (100 mm × 4.5 mm I.D.) and either chloroform ($CHCl_3$) or methanol (MeOH) was used as the eluent with a flow rate of 1 ml min^{-1}. Capacity factors (k') were calculated using the equation, $k' = (t_R - t_0)/t_0$, where t_R is the retention time and t_0 is the time to elute a void marker.

combinations of R^2 and R^3, suggesting poorer recognition-site-definition in regions corresponding to hydrophobic portions of the imprint template.[5]

When the eluent was changed from chloroform to methanol, no retention was observed for barbiturates. This means that hydrogen bonding is essential and aprotic solvents should be used in the system. It has previously been reported[6] that the malonylurea structure forms multiple hydrogen bonding to 2,6-alkylamidopyridine derivatives, suggesting that the barbiturates bind to the residues **1** in the imprinted polymer through multiple hydrogen bonding (Fig. 1).

Recently, artificial receptors have been reported for the recognition of barbiturates.[6,7] Although these artificial receptors could bind barbiturates selectively, their synthesis was tedious and time-consuming, requiring precise design of the size and shape of the receptor binding sites to align complementary regions. In contrast, complementary alignment is easily achieved using the molecular imprinting technique with a simple procedure involving self-assembly of template-functional monomer complex, followed by polymerization, enabling molecular recognition polymers to be prepared without detailed molecular design and multi-step synthesis.

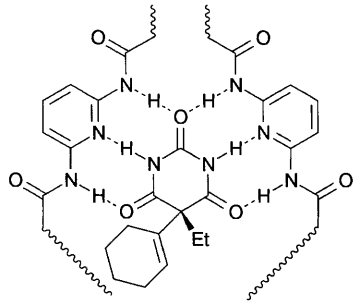

Fig. 1 Possible structure of the binding sites for cyclobarbital in the molecularly imprinted polymer

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