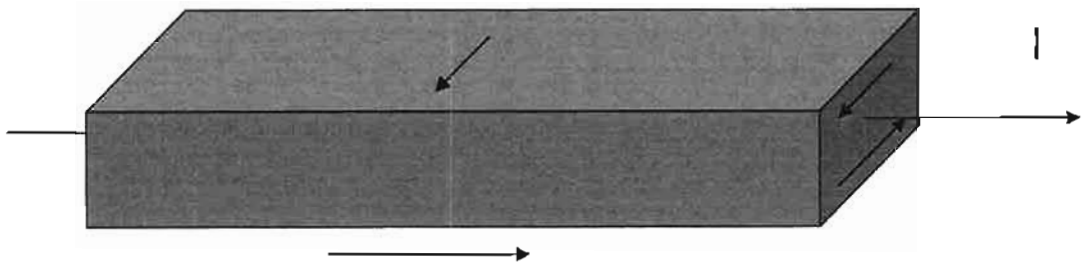
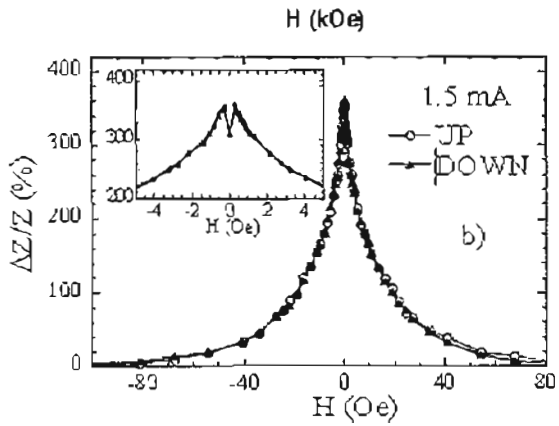
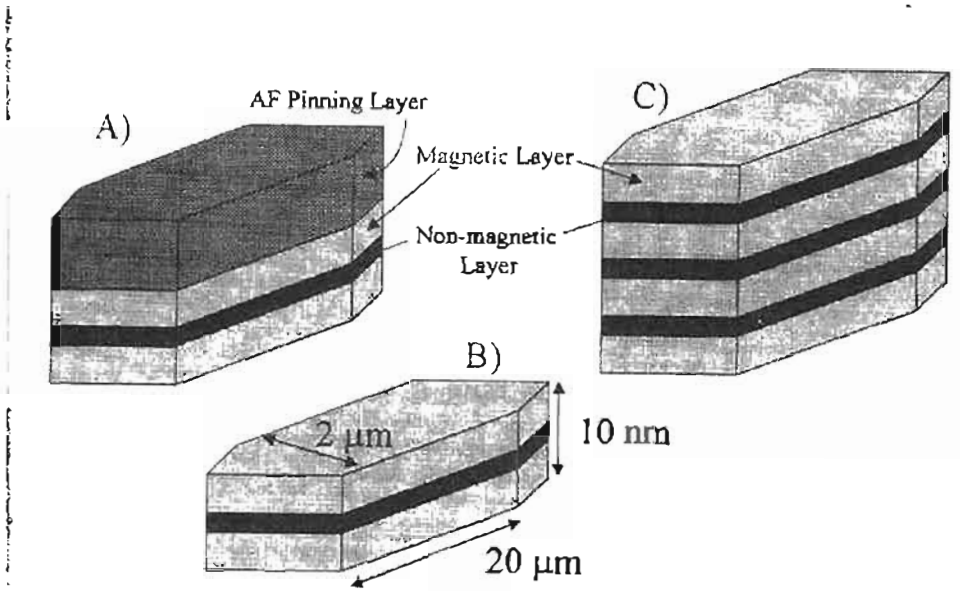
Magnetoimpedancia

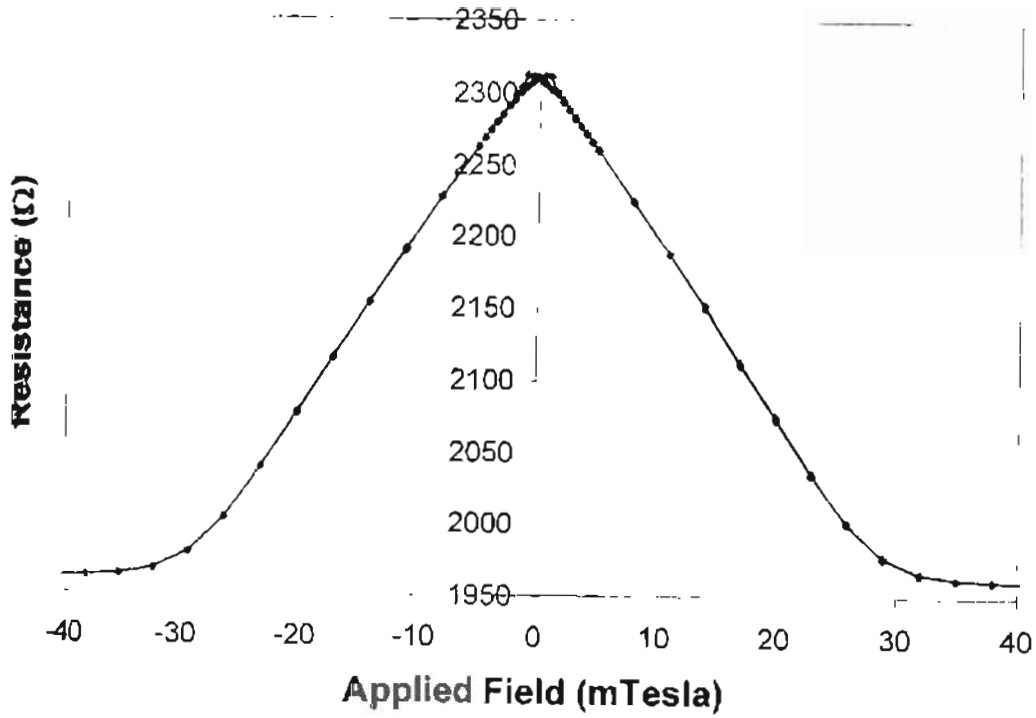


Magnetoimpedance
(MI, SI, GMI)





22 Cross sections of common GMR structures: (a) spin valve, (b) sandwich, and (c) multilayer.



27 GMR multilayer structure output: resistance versus field.

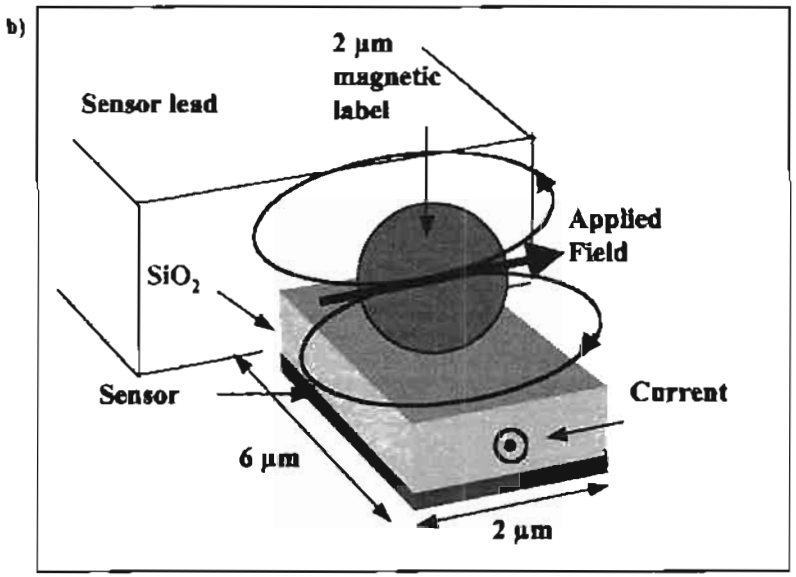
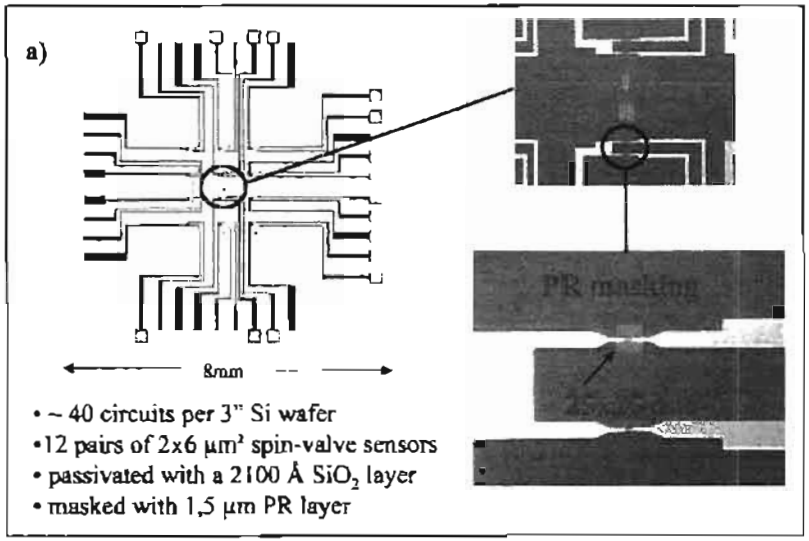


Fig 2 a) Cross-section of a MR-biochip, showing the spin valve sensor, the leads, the 2000Å thick SiO_2 functionalized passivation layer, the immobilized probes (in this case biotin), and the hybridized targets (streptavidin) coating the magnetic labels.The inset shows the top-view of the probe-pad. b) Detection geometry: a 15Oe in-plane field magnetizes the superparamagnetic labels, and in turn, these produce a transverse inplane field in the spin valve).



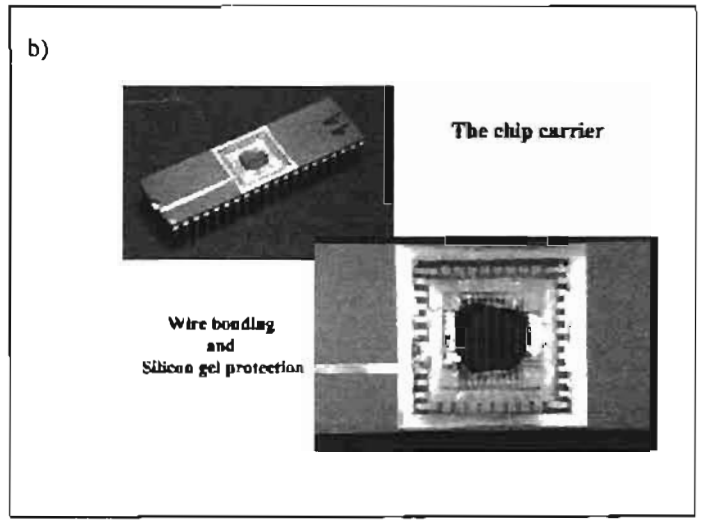
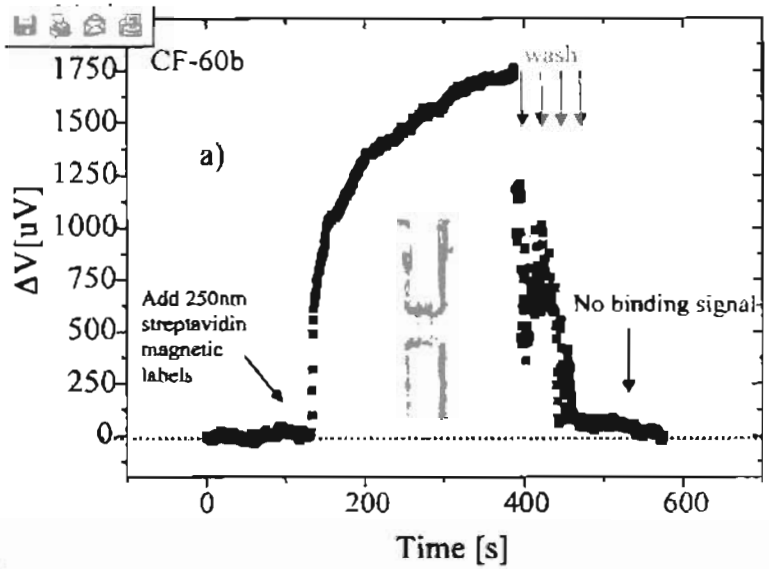
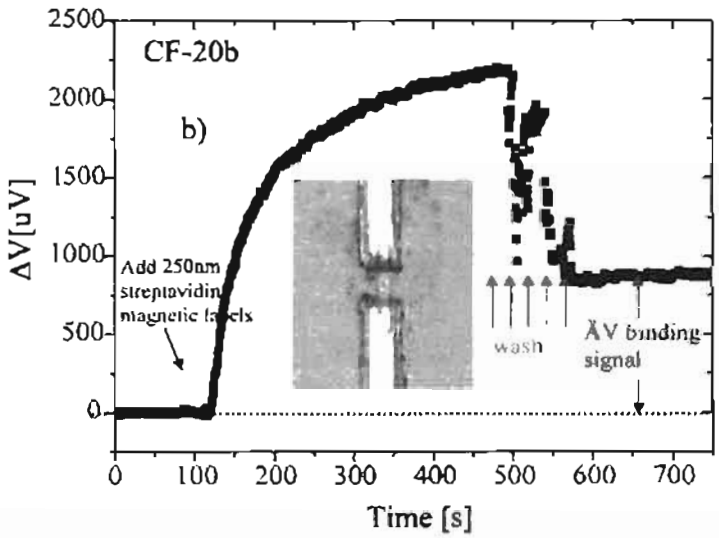


Fig 3 a) Schematics of one of the MR biochips, using differential detection, with one actl sensor and one reference sensor as two arms of a Wheatstone bridge configuration. b) Packaged MR biochips ready for biological assaying.





Metamateriales

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Curso Materiales Inteligentes

Fábrica Nacional de Moneda y Timbre- Real Casa de la Moneda

Junio 2010

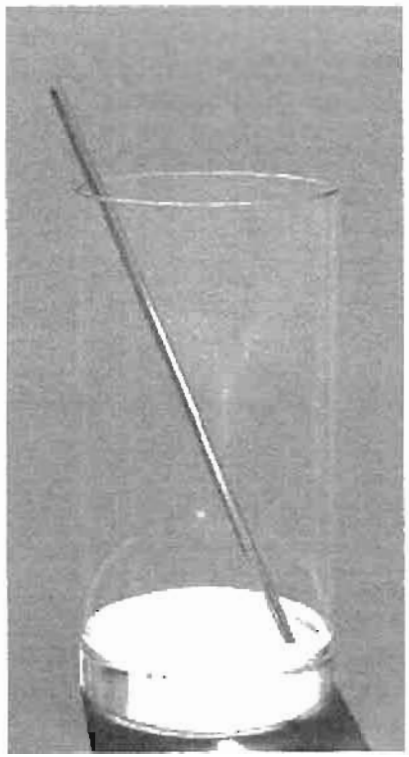
Indice

Indice

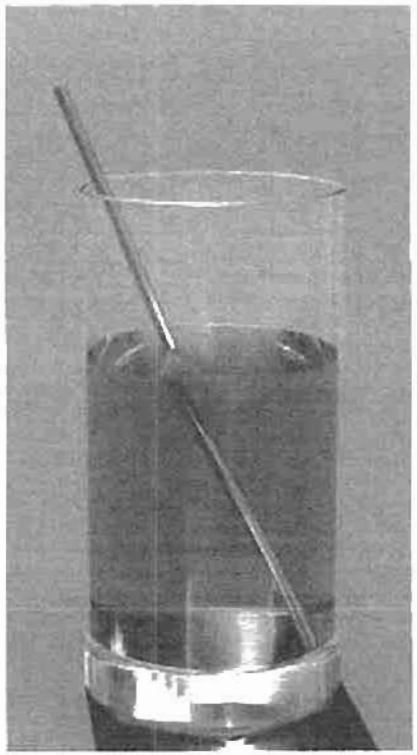
¿Qué es un MetaMaterial?

- "Meta" del griego μετα: "después/más allá"
- Ejemplo: *Metafísica* ("más allá de la física"): *rama de la filosofía que estudia los principios fundamentales de la realidad (más allá de la experimentación científica)*.
- Los **Metamateriales** son medios artificiales estructurados con propiedades (mecánicas, ópticas, eléctricas, magnéticas, etc) exóticas, es decir, que no se encuentran en los materiales ordinarios.
- En esta charla nos ocuparemos de los **Metamateriales Electromagnéticos (Ópticos)**

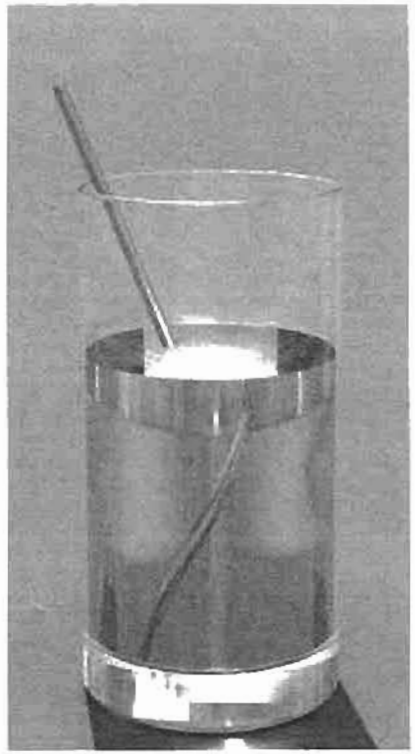
Ejemplo: Refracción Negativa



Vaso vacío



Agua
n = +1,3

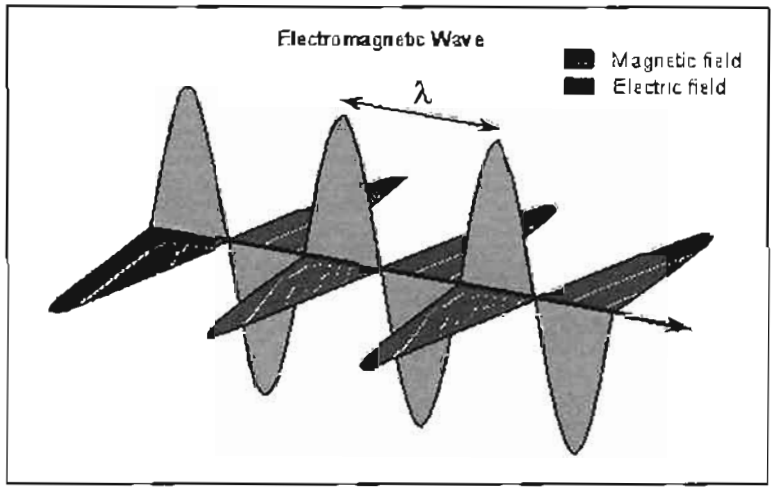


Metamaterial con n = -1,3

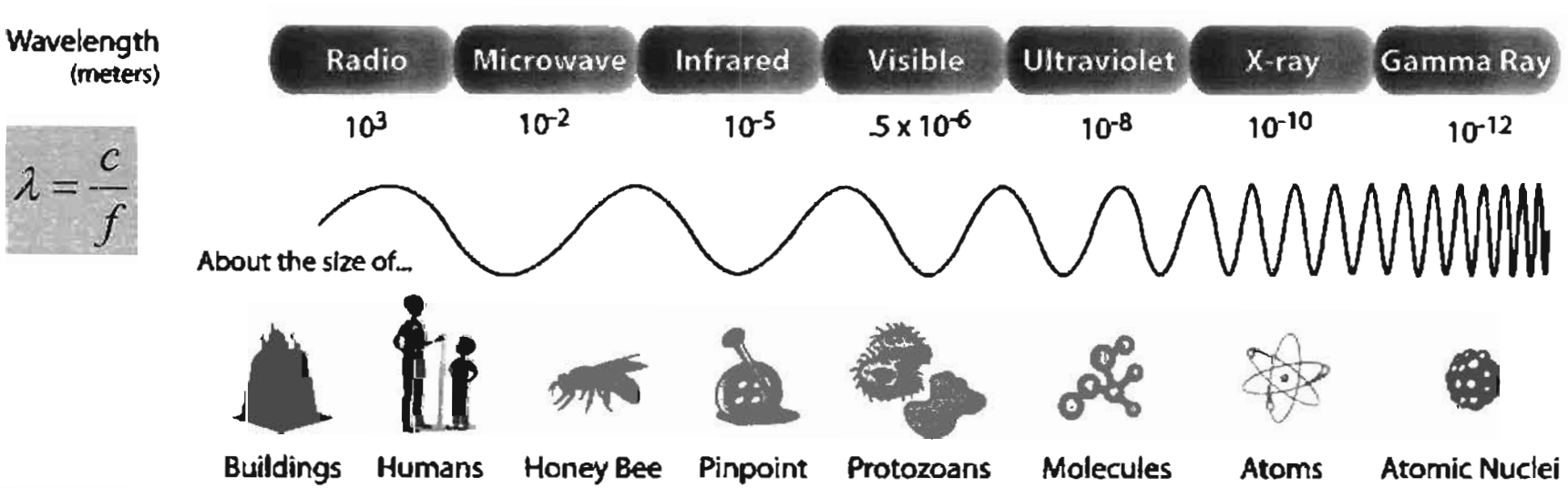
Indice

Radiación electromagnética

- Oscilaciones simultáneas de campos eléctricos y magnéticos que se propagan a la velocidad de la luz
- **Espectro Electromagnético**

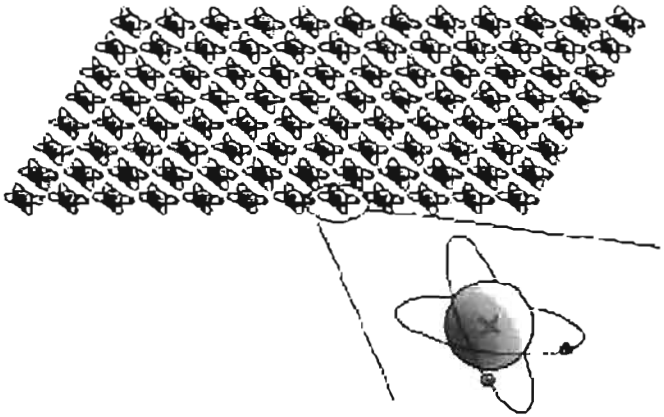


$$\lambda = \frac{c}{f}$$

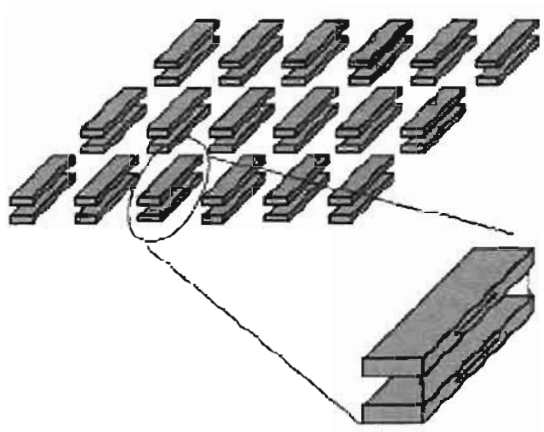


Propiedades ópticas

- Características ópticas de un material = propiedades de sus unidades + la distribución espacial de éstas



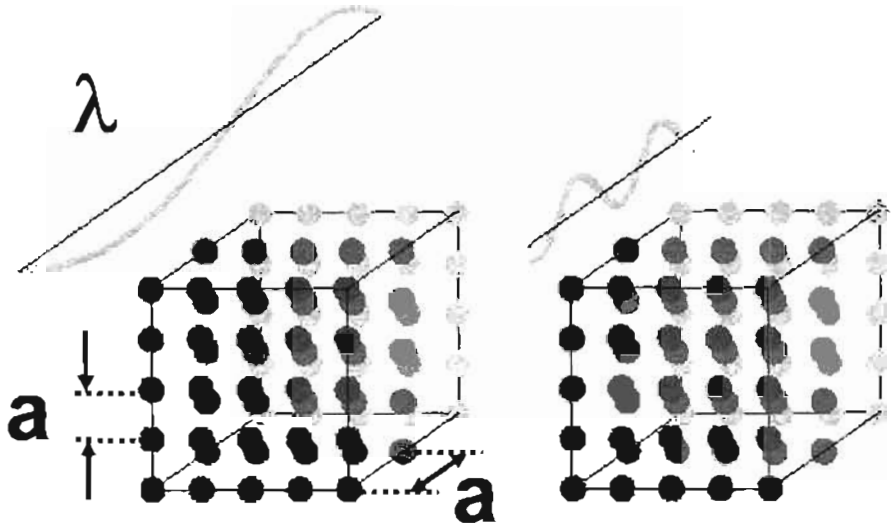
Material ordinario:
Unidades = átomos



Shalaev, *Opt. Lett.* (2005)

Metamaterial:
Unidades = pares de barras metálicas:
"átomos artificiales"

Propiedades ópticas

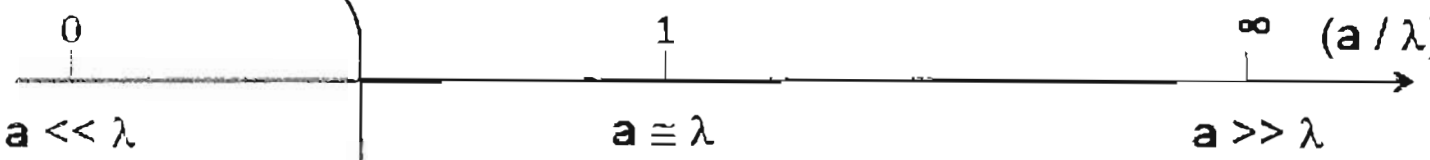


λ ≡ longitud de onda de la luz

a ≡ constante de red (**escala**)

- **a << λ** → teoría del medio efectivo (homogéneo)
- **a ~ λ** → efectos fotónicos - interferométricos
- **a >> λ** → elementos discretos

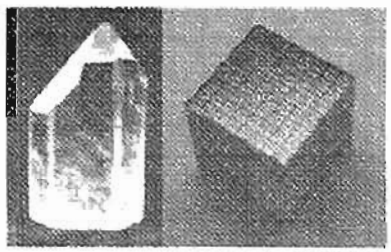
Efecto de la escala



Properties determined by ε and μ. Maxwell equations

Example:

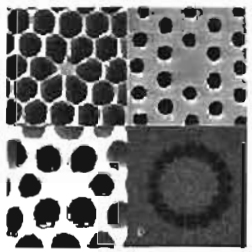
Glasses, Crystals
Metamaterials



Structure dominates. Properties determined by diffraction and interference

Example:

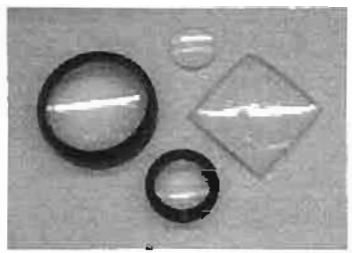
Photonic Crystals



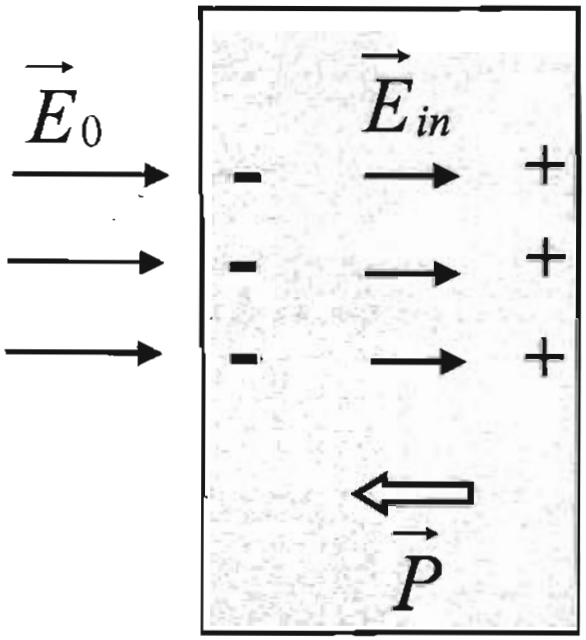
Properties described by geometrical optics and ray tracing

Example:

Lenses, Windows…



Permitividad eléctrica ε

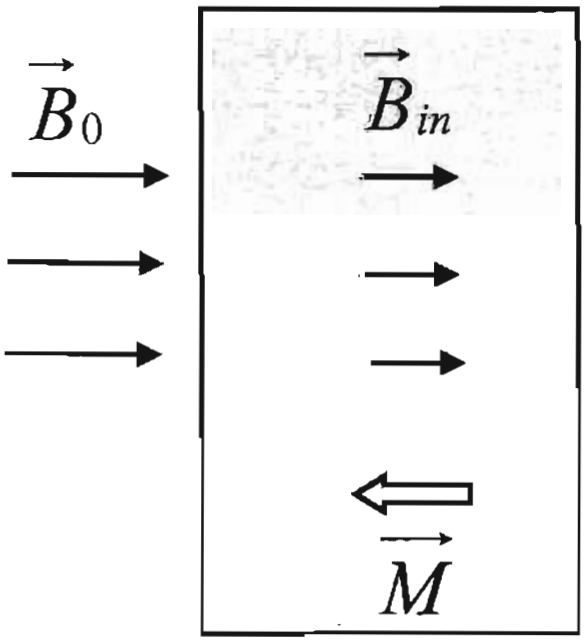


- E_0 campo eléctrico externo
- E_{in} campo eléctrico en el material

$$\varepsilon = \frac{E_0}{E_{in}}$$

- Mayoría de los materiales $\varepsilon > 1$ (ej. $\varepsilon = 2{,}25$ para vidrio)
- Excepciones: ε **< 0 en metales** (visible, IR,…)
- La permitividad depende de la frecuencia $\varepsilon = \varepsilon(\omega)$

Permeabilidad magnética μ



- B_0 campo magnético externo
- B_{in} campo magnético en el material

$$\mu = \frac{B_0}{B_{in}}$$

- Materiales ferromagnéticos (Fe, Ni) $\mu >> 1$
- Materiales paramagnéticos ("no magnéticos") $\mu = 1$
- La permeabilidad depende de la frecuencia $\mu = \mu(\omega)$

Indice de refracción

➢ La propagación de las ondas electromagnéticas en un medio está determinada por ε y μ del medio:

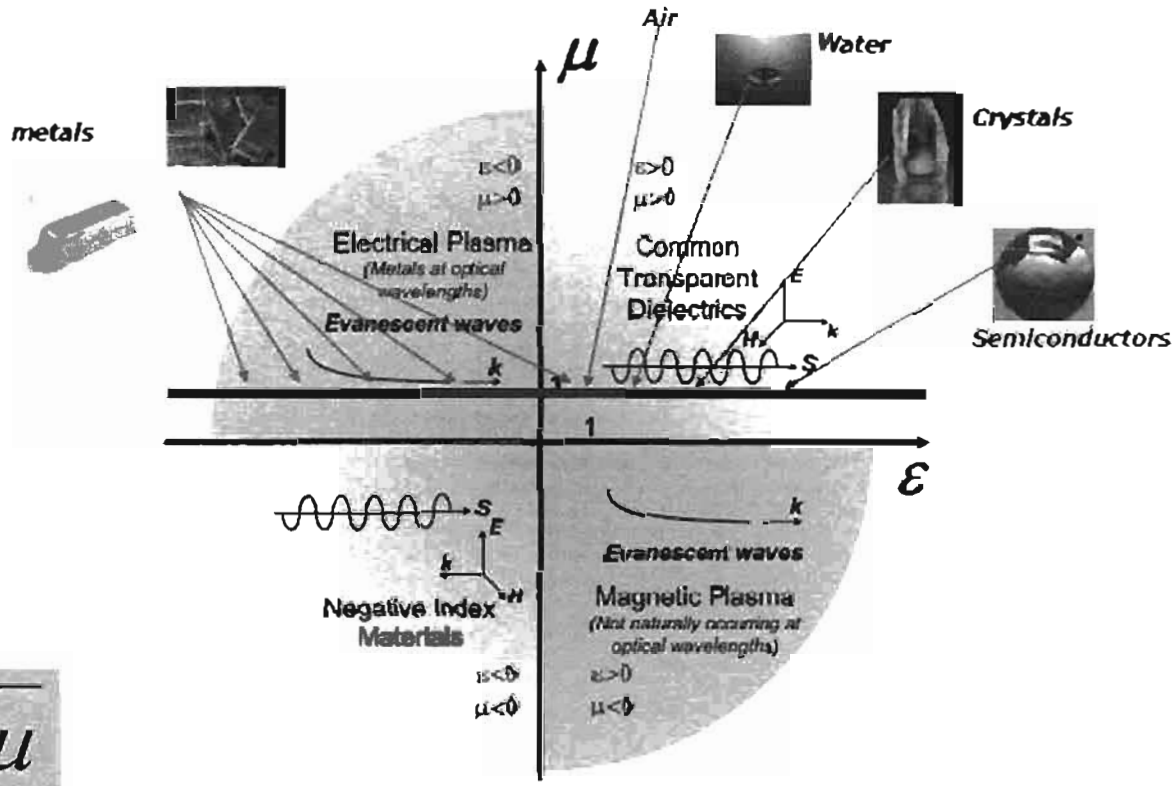
$$v = \frac{c}{\sqrt{\varepsilon\mu}} = \frac{c}{n}$$

$v \equiv$ velocidad de la onda

$c \equiv$ velocidad de la luz en el vacío

$n \equiv$ índice de refracción del medio

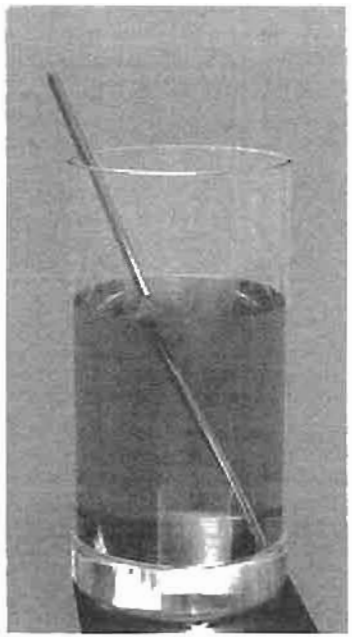
$$n = \pm\sqrt{\varepsilon\mu}$$

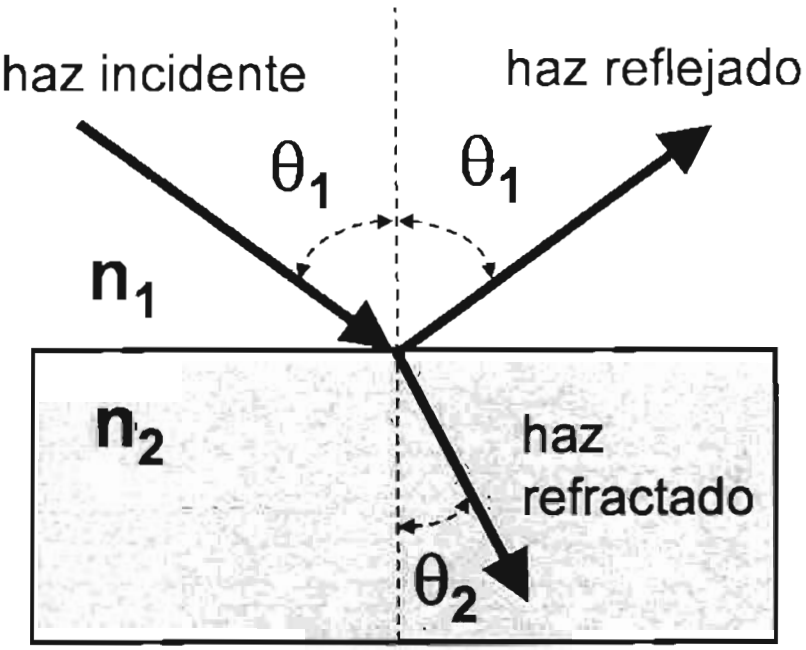


Indice de refracción

- Ley de refracción o Ley de Snell

$$n_1 sen\theta_1 = n_2 sen\theta_2$$





- Materiales ordinarios $n_1, n_2 > 0$ (positivos)

Metamateriales

1- Introducción

2- Propiedades electromagnéticas de los materiales

3- Metamateriales Electromagnéticos

4- Fabricación de Metamateriales Electromagnéticos

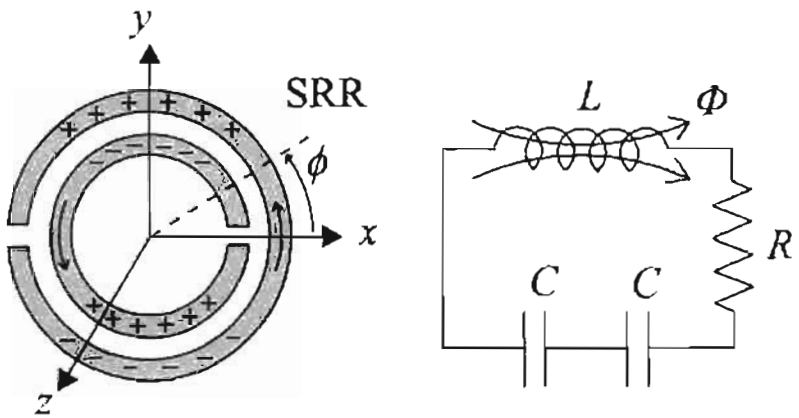
5- Aplicación: Elementos de Seguridad

6- Conclusiones

Respuesta magnética

- "Átomos magnéticos": unidades básicas con $\mu \neq 1$ a ciertas frecuencias
- Anillo metálico resonador partido → Resonador LC (bobina+condensador)

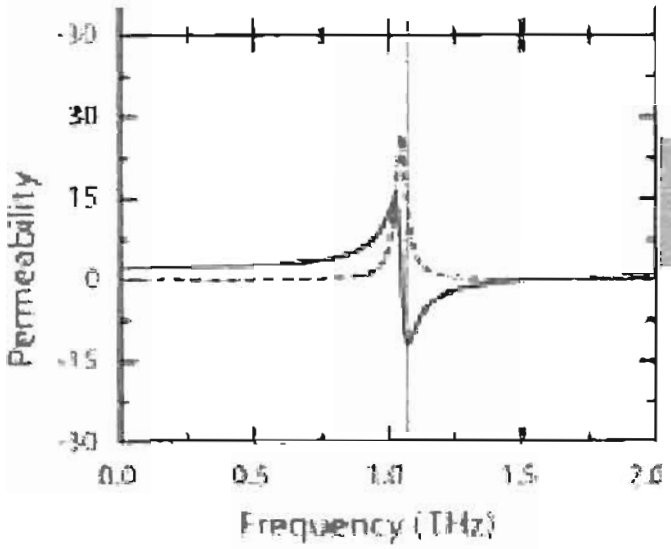
Pendry, *IEEE Trans. Microwave Theory Tech.* (1999)



(a) (b)

Inductancia (L) y Capacidad (C) dependen de la **geometría**

Frecuencia de resonancia $\omega_0 \approx \sqrt{1/LC}$

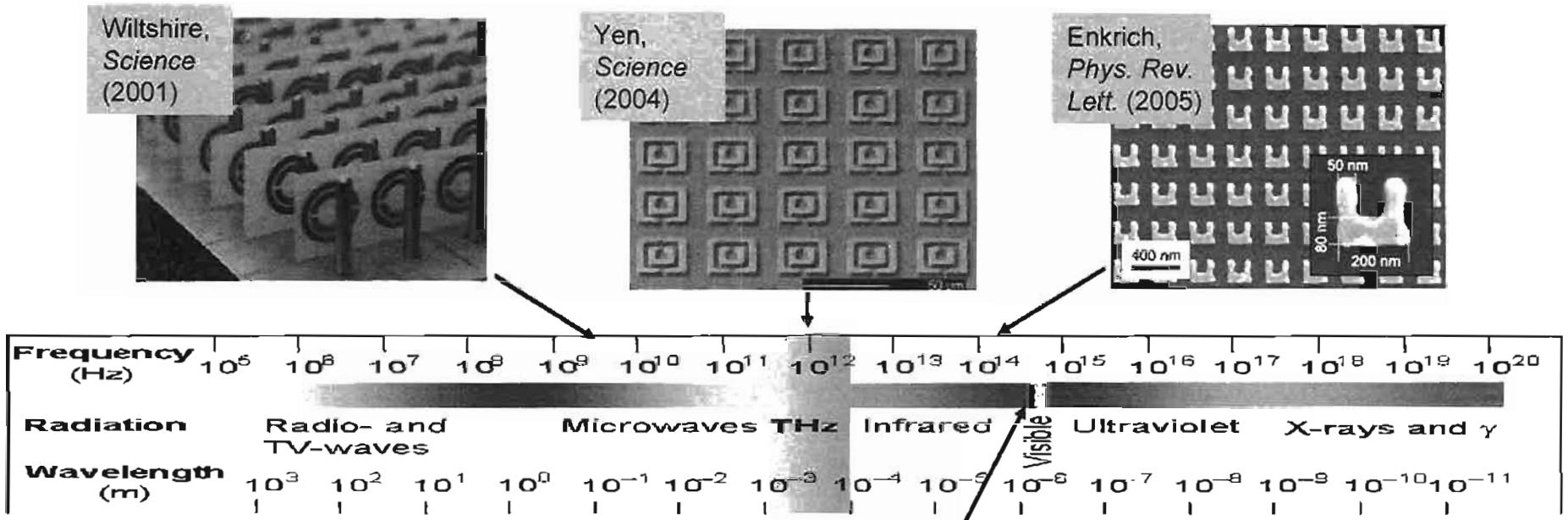


Padilla, *Materials Today* (2006)

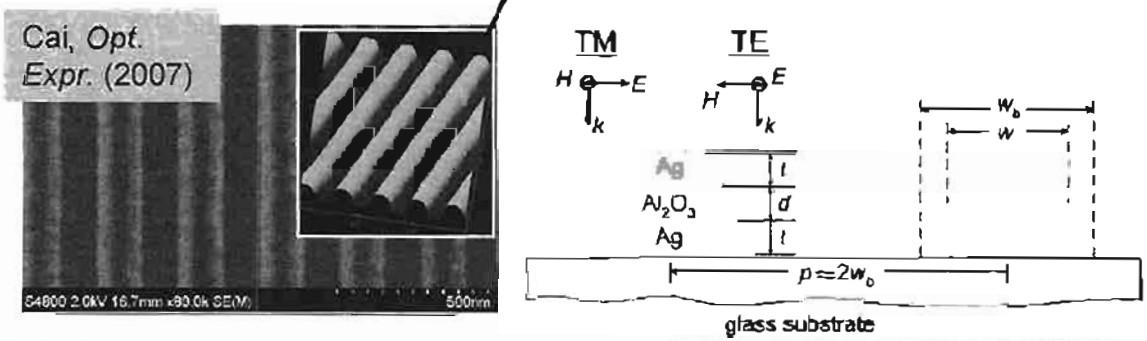
- $\mu \neq 1$ alrededor de ω_0
- μ es muy negativo para $\Delta\omega > \omega_0$

Metamateriales magnéticos

- Estructura diseñada para tener $\mu_{efectiva} \neq 1$ a determinadas frecuencias



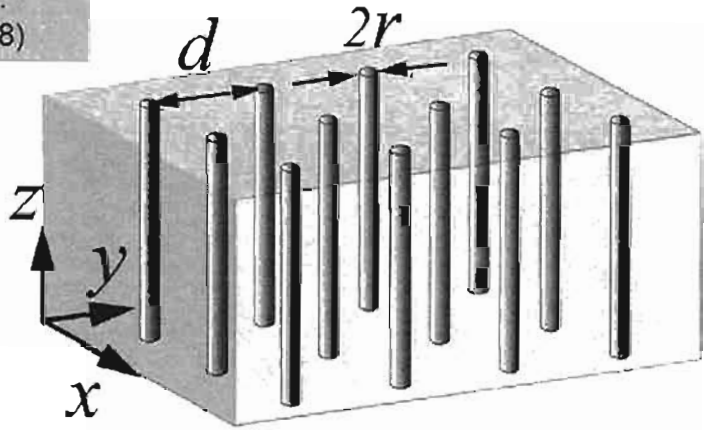
- Metamaterial magnético en el visible (luz roja)

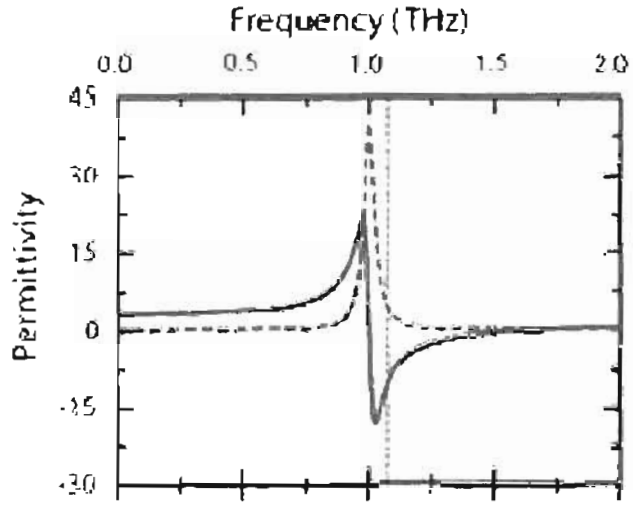


Respuesta eléctrica

- Hilos metálicos = "átomos eléctricos" con resonancias en $\varepsilon(\omega)$ determinadas por la geometría (longitud y espesor de los hilos)

Liu, *Opt. Expr.* (2008)





Padilla, *Materials Today* (2006)

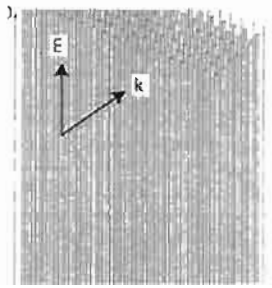
- Metamaterial eléctrico = estructura diseñada para tener una $\varepsilon_{efectiva}$ concreta a determinadas frecuencias

Indice de refracción Negativo

$$n = \pm\sqrt{\varepsilon\mu}$$

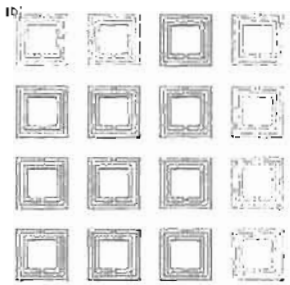
- Victor Veselago (1968): $\varepsilon < 0\,,\ \mu < 0 \rightarrow n < 0$
- Materiales "naturales" con n<0 no encontrados hasta la fecha
- Metamateriales pueden ser diseñados para tener n<0

MM eléctrico
$\varepsilon(\omega) < 0$



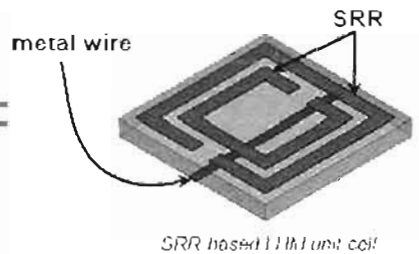
+

MM magnético
$\mu(\omega) < 0$



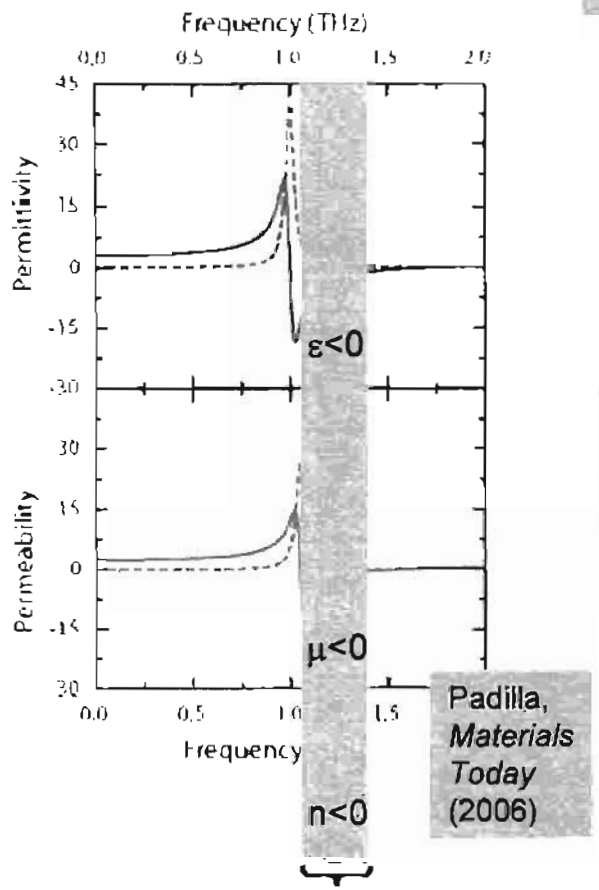
=

MM electromagnético
n (ω) < 0

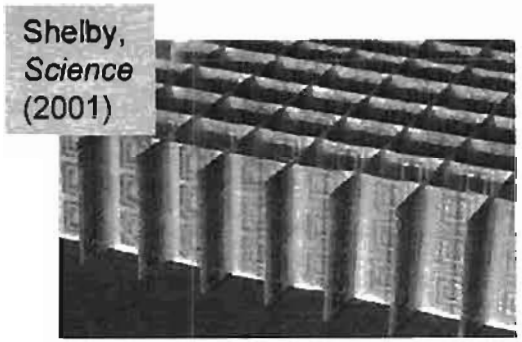


Indice de refracción Negativo

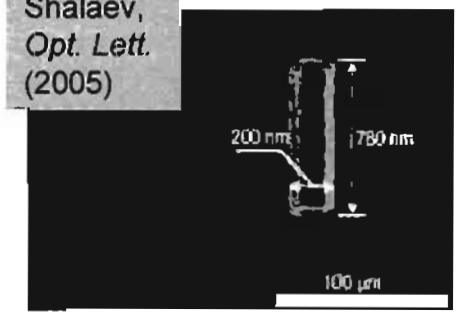
$$n^2(\omega) = \varepsilon(\omega)\cdot\mu(\omega)$$



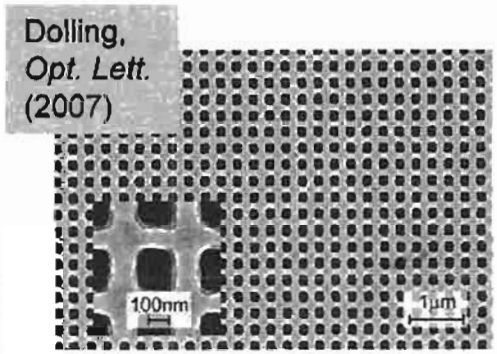
banda espectral $\Delta\omega$



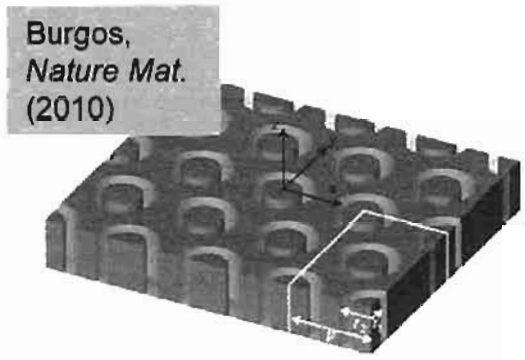
Microondas



Banda comunicaciones fibra óptica



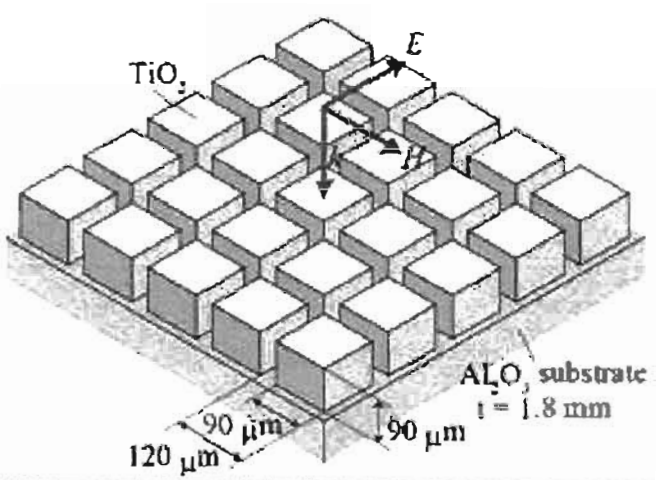
Visible (rojo)

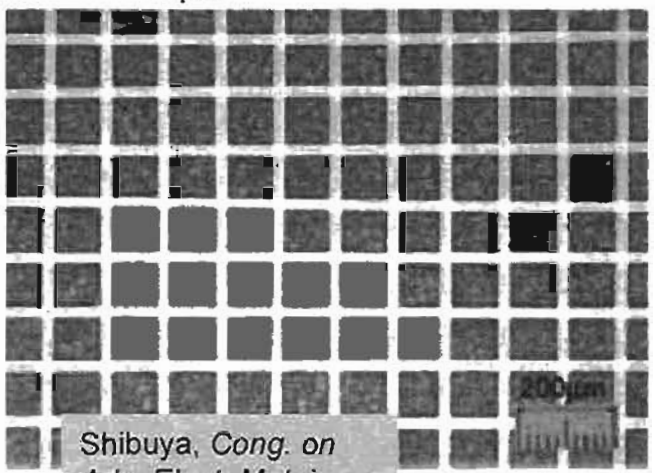


Visible (azul)

MMs dieléctricos

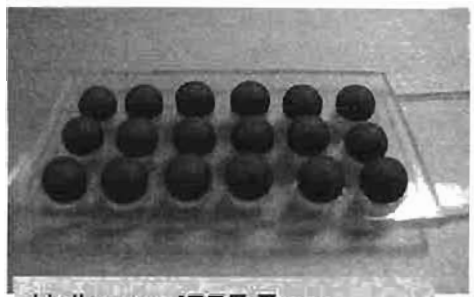
- *Metales* → *pérdidas ópticas* → degradación de la respuesta del MM
- **Alternativa**: *MM dieléctrico*: matriz dieléctrica (ε_1,μ_1) con incrustaciones (partículas) de otro dieléctrico (ε_2, μ_2) → *Resonancias en partículas dieléctricas*: $\varepsilon_{ef}(\omega)$, $\mu_{ef}(\omega)$ Zhao, *Materials Today* (2009)
- Se precisan dieléctricos con altas permitividades (ε) y bajas pérdidas





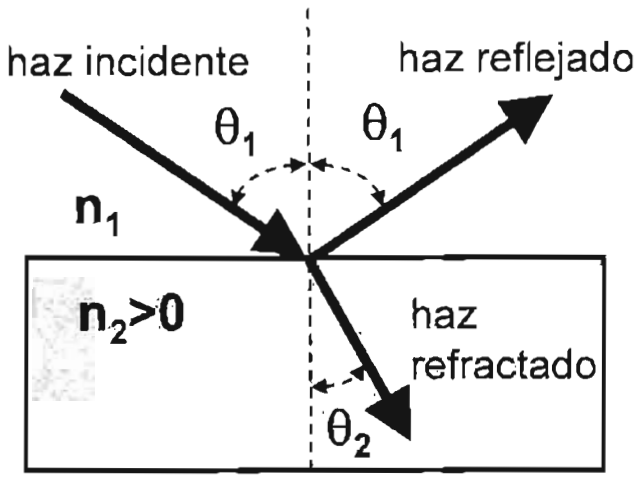
Shibuya, *Cong. on Adv. Elect. Mat. in Microw. Opt.* (2008)

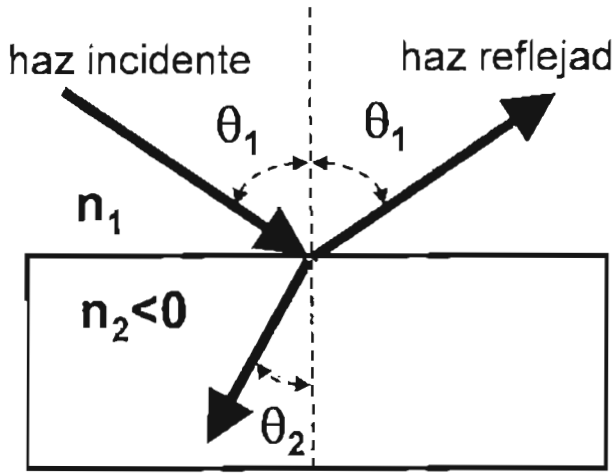
Red de partículas magneto-dieléctricas



Holloway, *IEEE Trans. Antennas Propagat.* (2003)

Indice de refracción Negativo



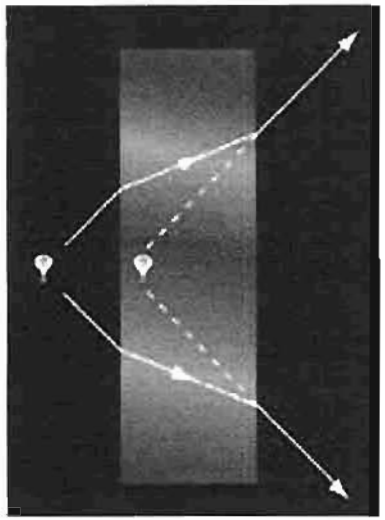


Ley de Snell

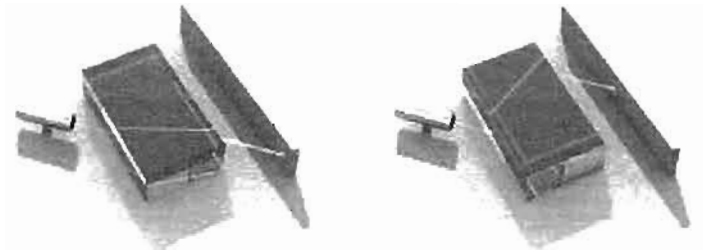
$$n_1 sen\theta_1 = n_2 sen\theta_2$$

Aplicaciones de los MMs

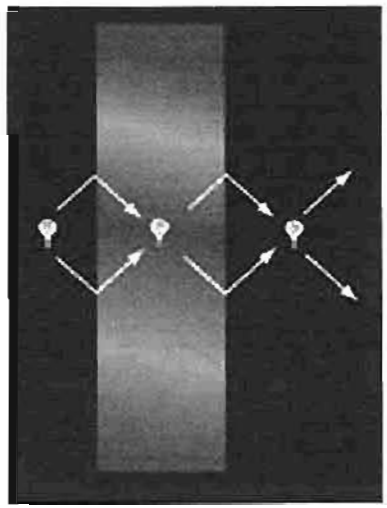
n > 0



Luz saliente es desviada



n < 0

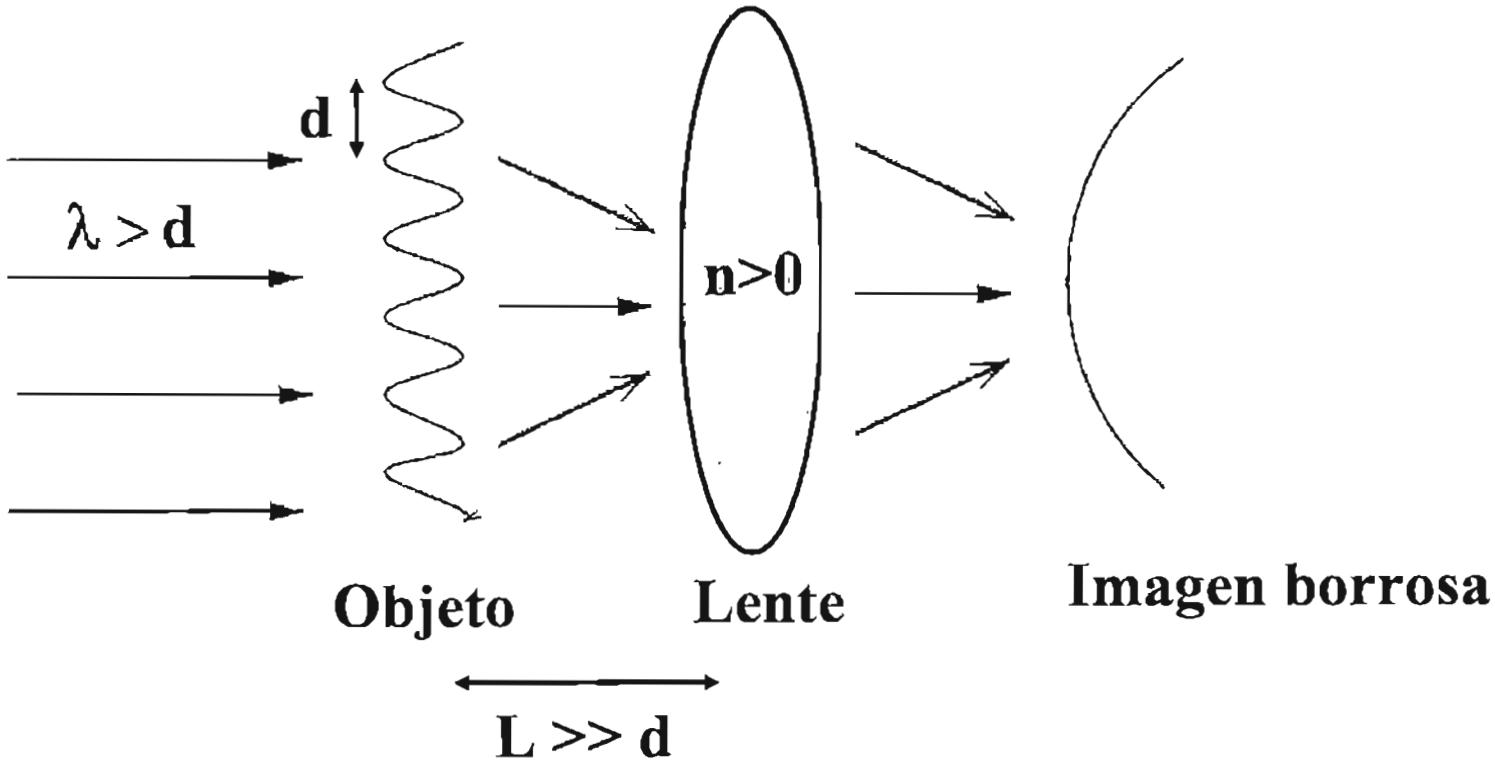


Luz saliente es ENFOCADA

SUPERLENTE ⟶ lente con **Super-Resolución**

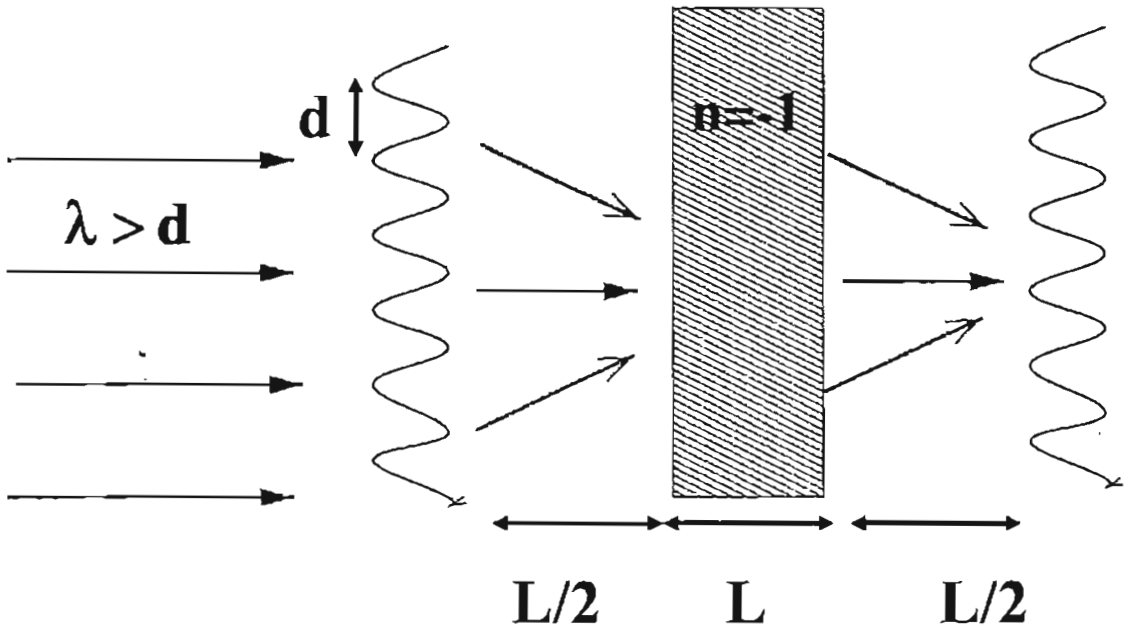
Resolución de una lente

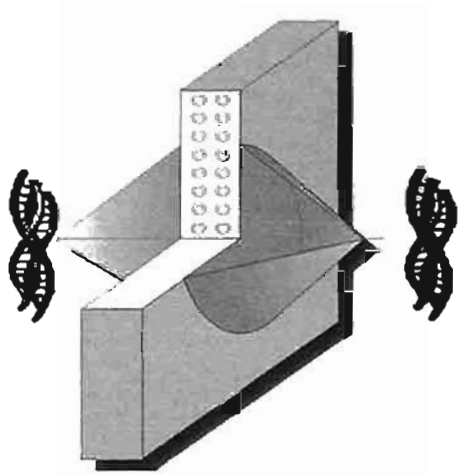
- Lente convencional (n>0) ⟶ Máxima resolución d ~ λ (límite difracción)
- d < λ ⟶ se pierden los motivos más pequeños del objeto



Superlente

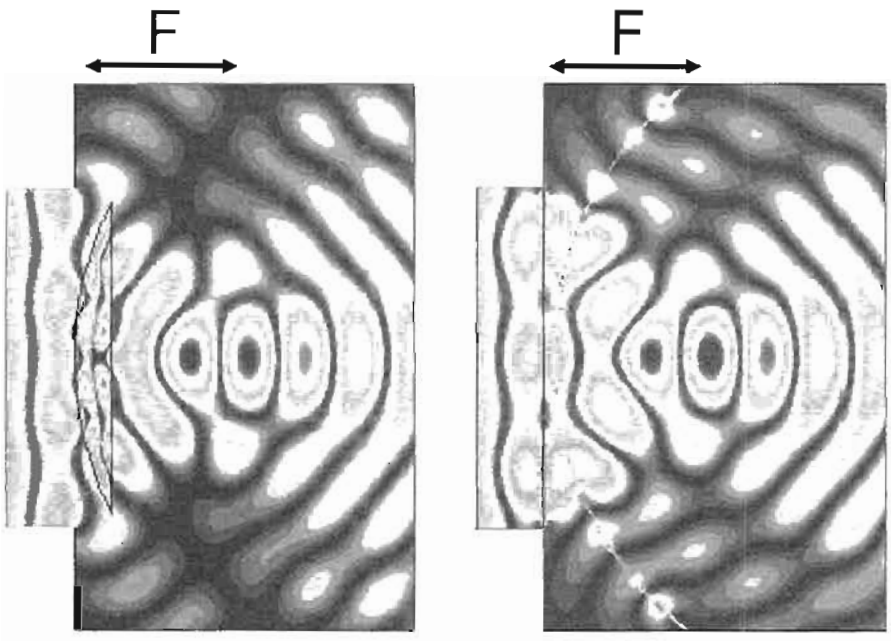
- Lámina con **n = -1** (ε= -1, μ= -1) $\rightarrow$ **SUPERLENTE PLANA**
- **Resolución < λ** $\rightarrow$ evita la degradación de la imagen





Smith, *Science* (2004)

Superlente



$n = 3$ $n = -1$

$$F = \frac{R}{|n-1|}$$

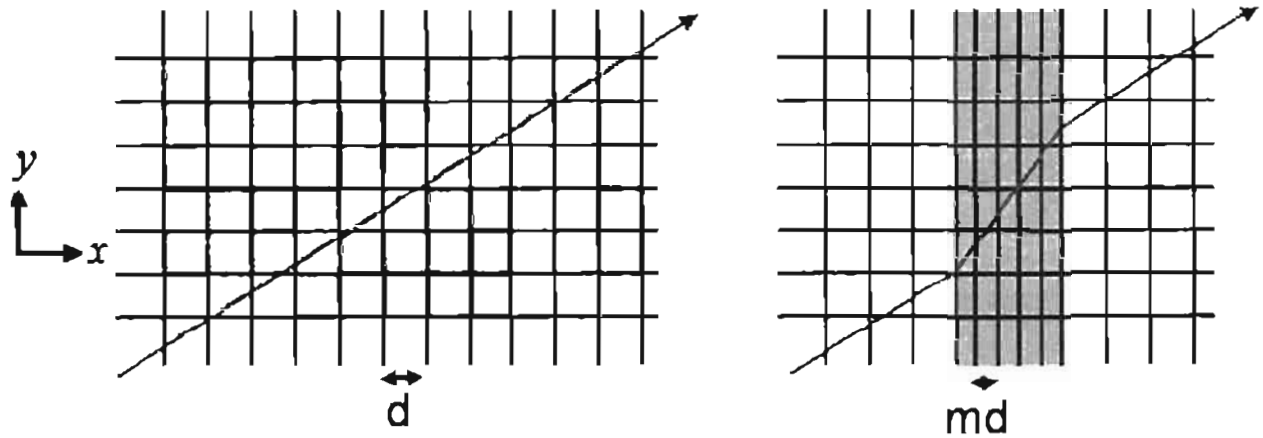
F= distancia focal

R= radio de curvatura

- Lente de foco corto: se necesita un material "convencional" con un índice muy grande y/o un gran radio de curvatura
- Lente de índice negativo mucho mas delgada y menor índice

Guiando luz con MMs

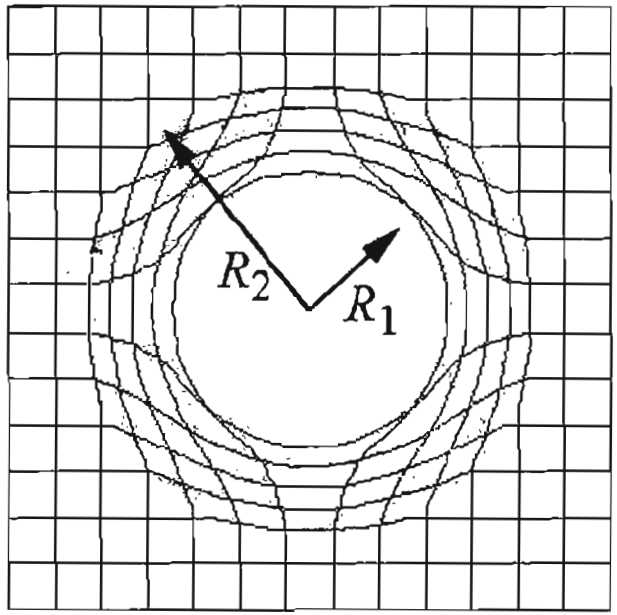
Transformación óptica

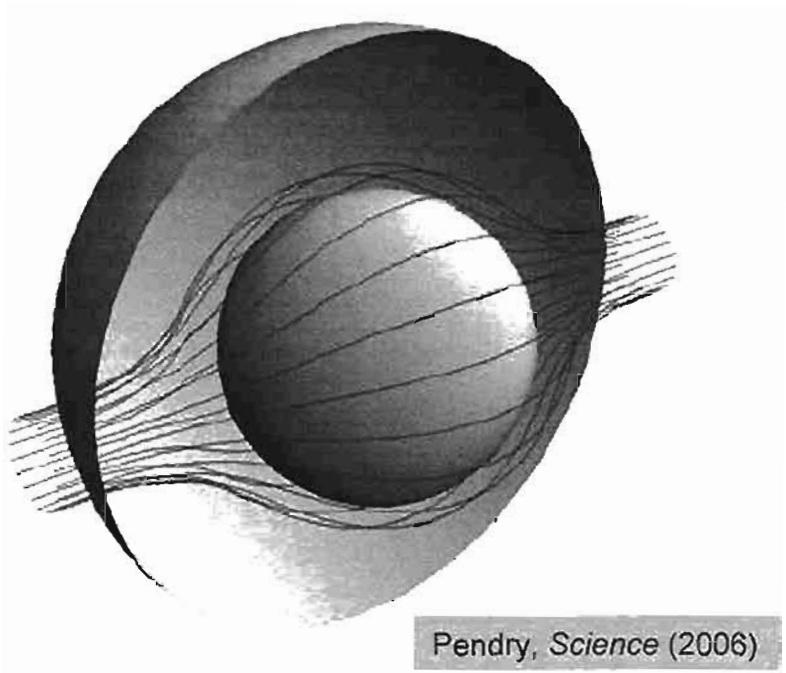


- Transformación de coordenadas en el eje x: **d** → **m·d**
- Trayectoria del rayo es modificada en la región comprimida
- Nuevos valores de **ε(x,y)** y **μ(x,y)** → **Metamaterial**

Capa de Invisibilidad

➢ Una forma de invisibilidad: modificar las trayectorias de los rayos de luz haciendo que rodeen un objeto sin producir sombras





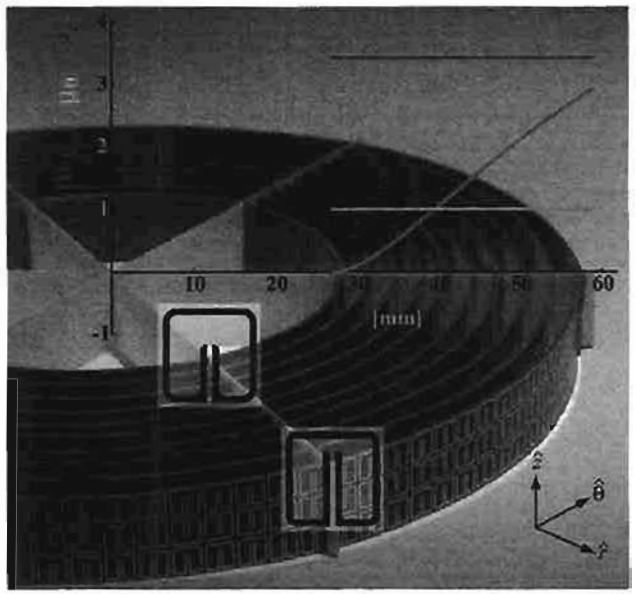
Pendry, *Science* (2006)

➢ Transformación de coordenadas
→ **Metamaterial con** $\varepsilon(\mathbf{r},\theta,\phi)$, $\mu(\mathbf{r},\theta,\phi)$

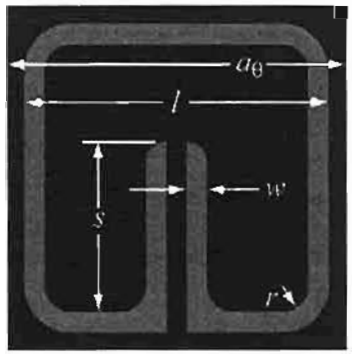
➢ **Capa de Invisibilidad**:
Los rayos rodean la zona cubierta

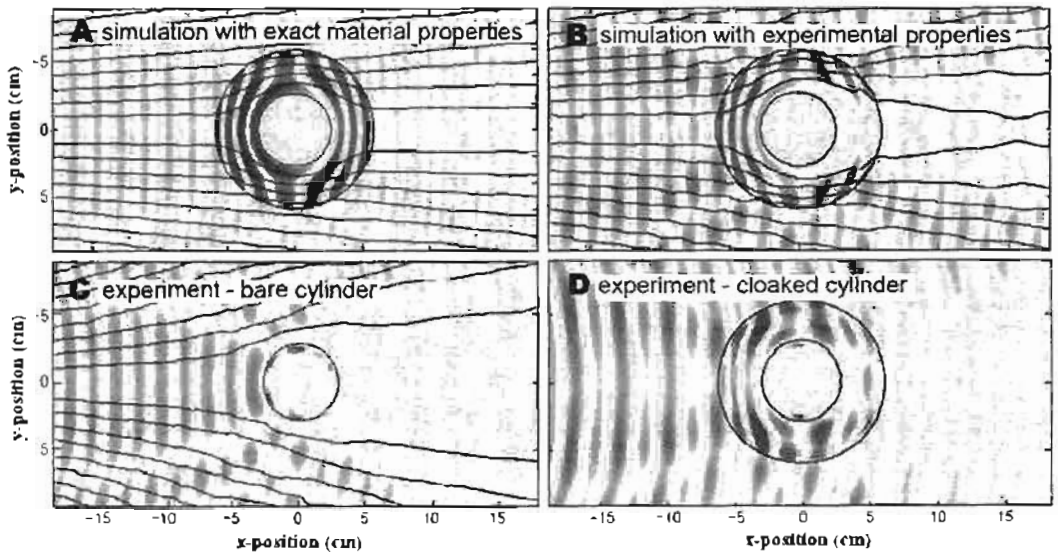
Capa de Invisibilidad

- Capa de Invisibilidad con MMs demostrada en rango de microondas



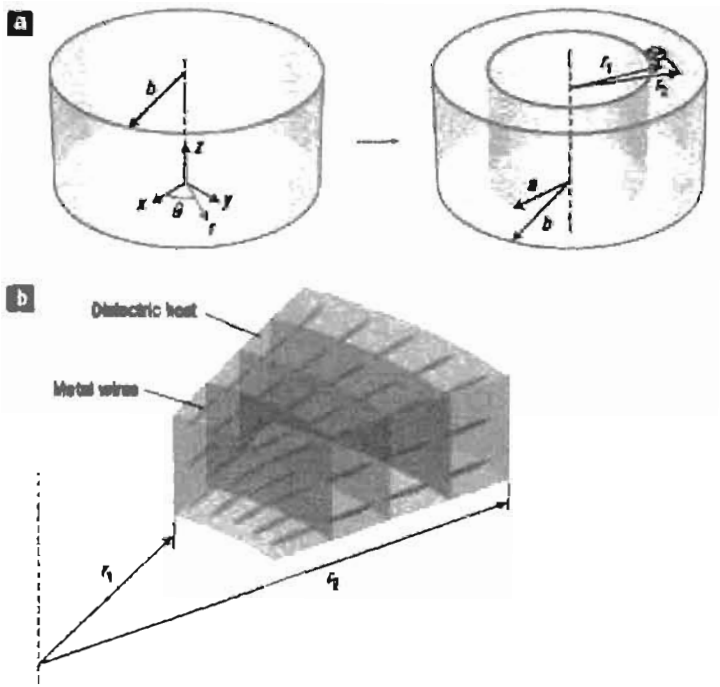
Schurig, *ScienceExpress* (2006)

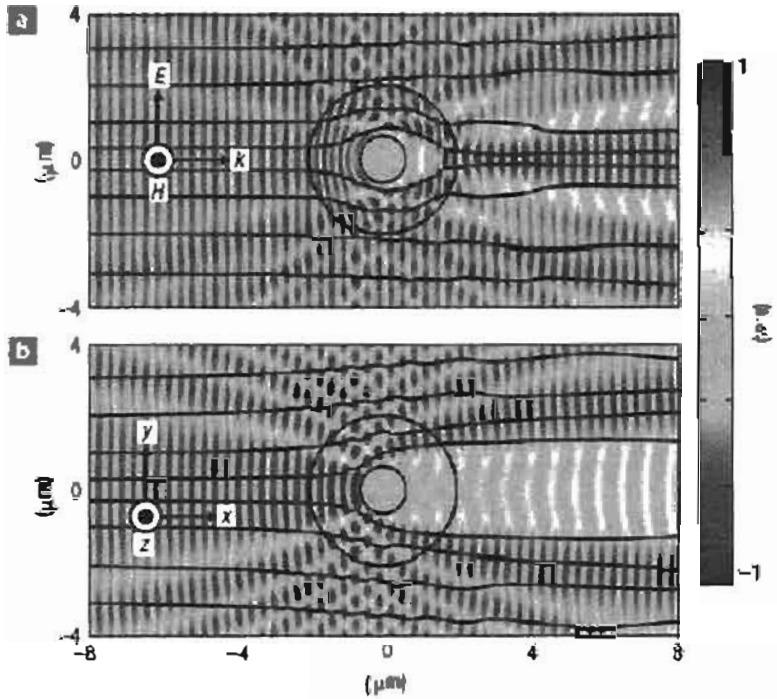




Capa de Invisibilidad

- "Auténtica" invisibilidad deber ocurrir en el espectro visible. De momento, sólo teóricamente





Capa de invisibilidad en el rojo (λ= 633 nm)

Cai, *Nature Photonics* (2007)

Indice

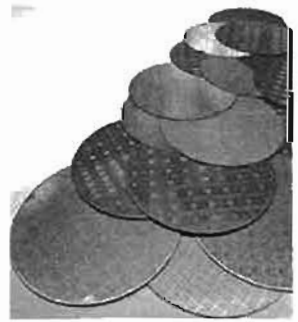
Materias Primas

- Metales (cobre, plata, oro,…)

  Dieléctricos (vidrios, plásticos, cerámicas,…)

  Semiconductores (GaP, Ge, SiC, …)

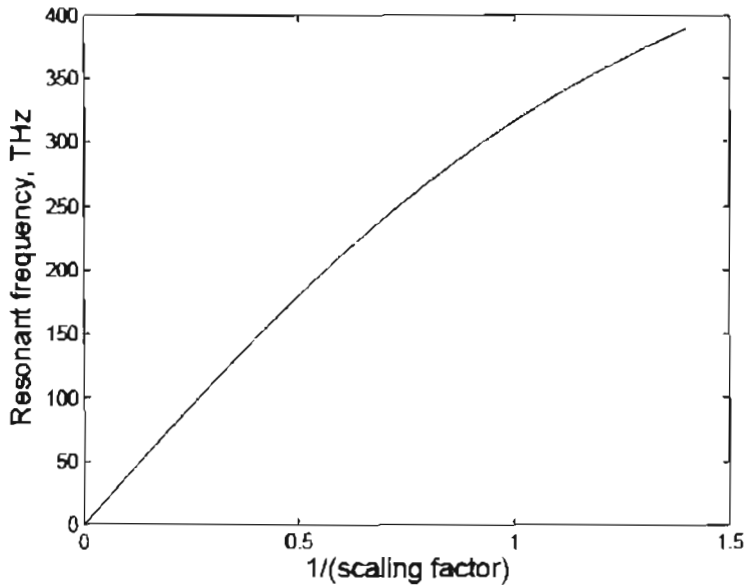
- Características a las frecuencias de trabajo:
  - ε, μ
  - Bajas pérdidas (absorción)
  - Bajo coste y fácil manufactura



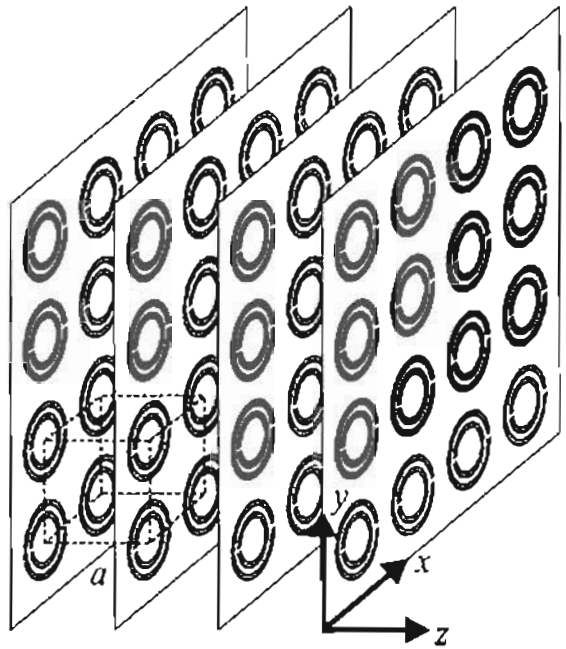
- Materiales activos (Semiconductores, moléculas orgánicas, tierras raras, …) → ganancia óptica → compensan pérdidas

Frecuencia-Escala

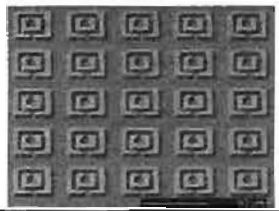
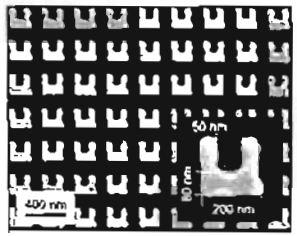
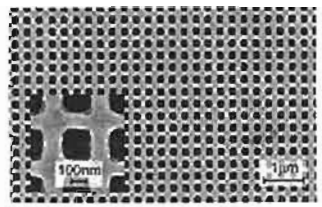
- ¿Qué rango espectral nos interesa? → Escala
- Recuerde: constante de red, a << λ



Klein, *Opt. Lett.* (2006)

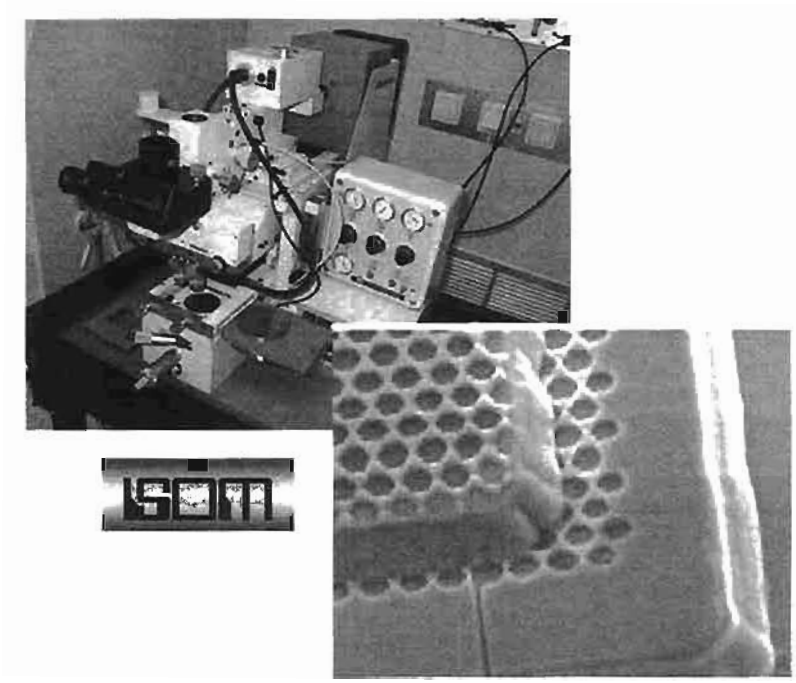


Frecuencia-Escala-Fabricación

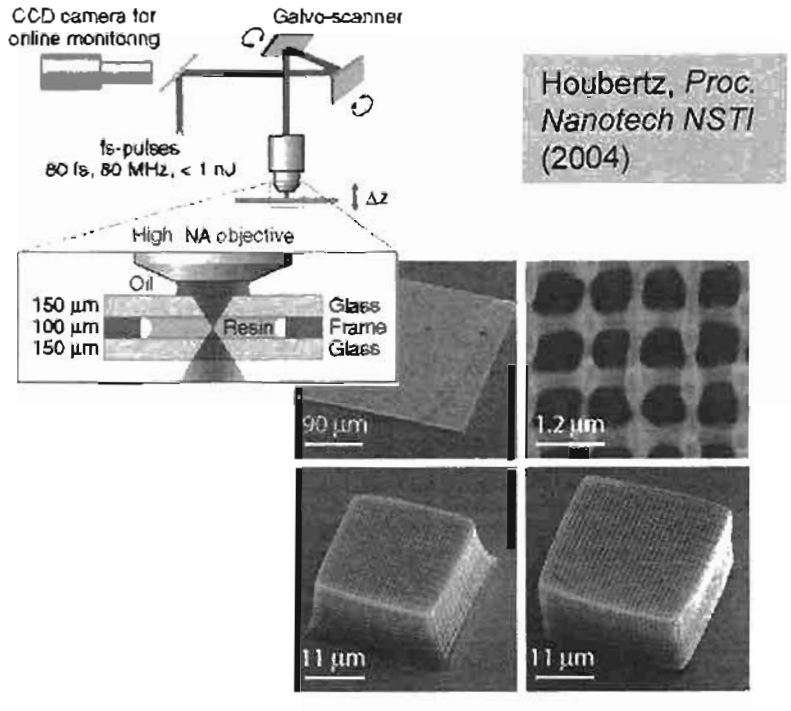
f, λ	a	Fabricación	
5 GHz, 6 cm *Microondas*	8 mm	Circuitos Impresos	 Wiltshire, *Science* (2001)
1 THz, 300 μm *Terahercios*	50 μm	Microtecnología	 Yen, *Science* (2004)
200 THz, 1,5 μm *IR cercano* *(comunic. fibra ópt.)*	300 nm	Nanotecnología	 Enkrich, *Phys. Rev. Lett.* (2005)
385 THz, 780 nm *Visible:Rojo*	250 nm	Nanotecnología	 Dolling, *Opt. Lett.* (2007)

Microfabricación

- Resolución > 1 micra (10^{-6} metros)



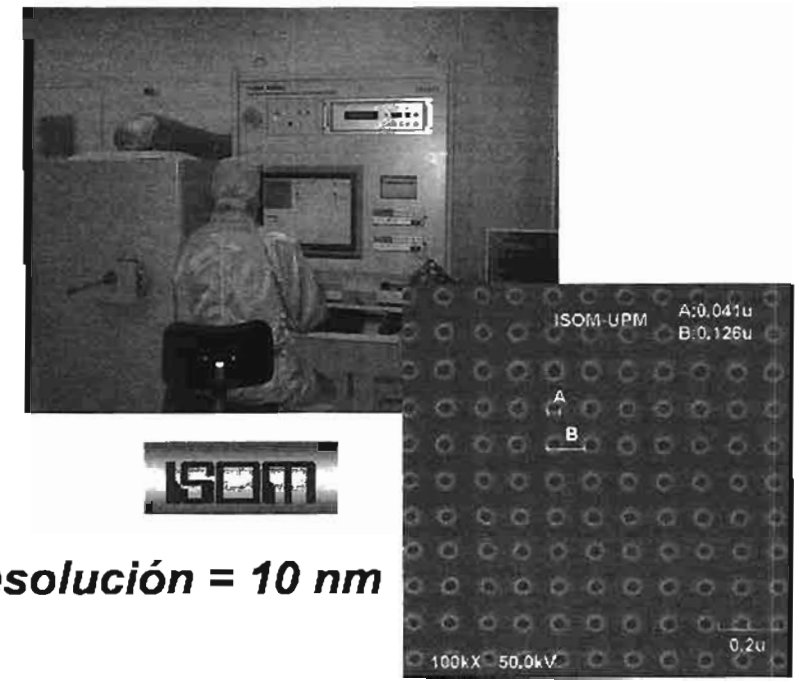
Fotolitografía con lámpara de ultravioleta (circuitos integrados)



Litografía láser por absorción (two-photon absorption)

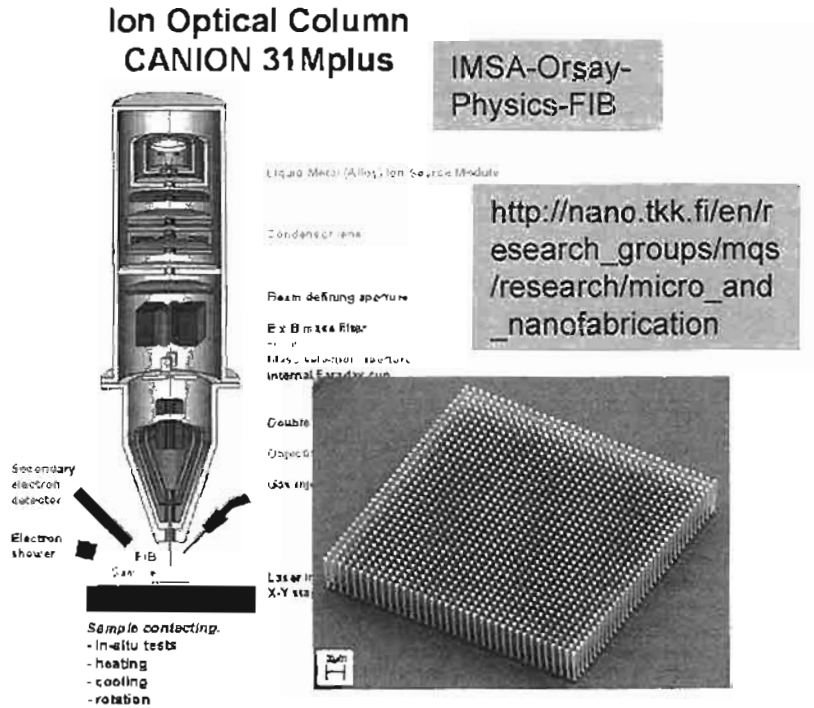
Nanofabricación

- Resolución < 1 micra (10^{-6} metros)



Resolución = 10 nm

Litografía por haz de electrones

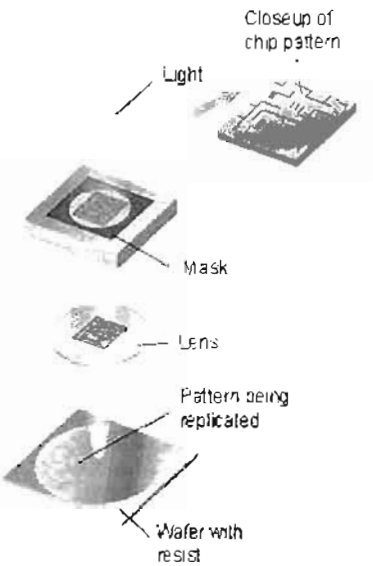


Escritura por haz de iones (FIB)

Técnicas de Nanofabricación

top-down

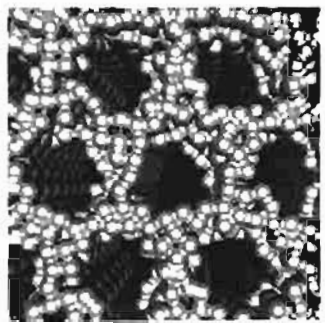
- Litografía y ataque sobre substrato
- Eliminan material selectivamente para crear estructuras
- Buena reproducibilidad
- Equipos caros



http://www.research.cornell.edu/KIC/events/Journalists2004/Bottom_Up_Fabrication.pdf

bottom-up

- Coloide, auto-ensamblado, catálisis química
- Añaden material selectivamente para crear estructuras
- Pobre reproducibilidad
- Procedimiento económico



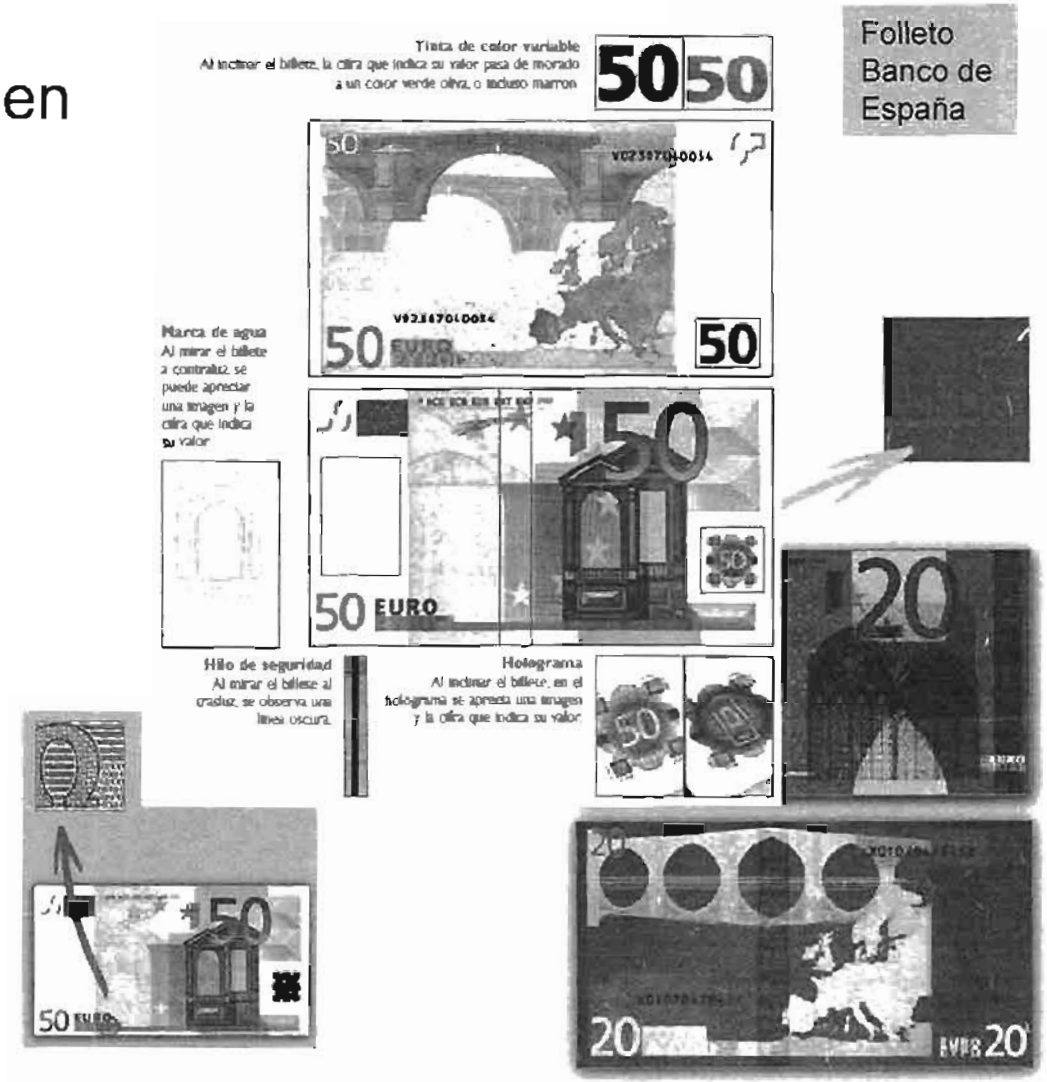
Scott Warren and Uli Wiesner, Cornell University

Indice

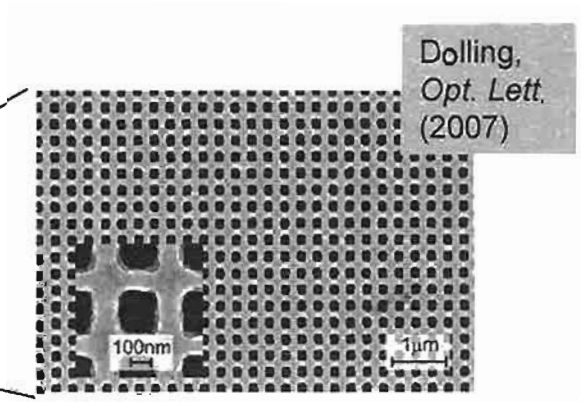
Seguridad óptica en billetes

Elementos de seguridad en billetes:

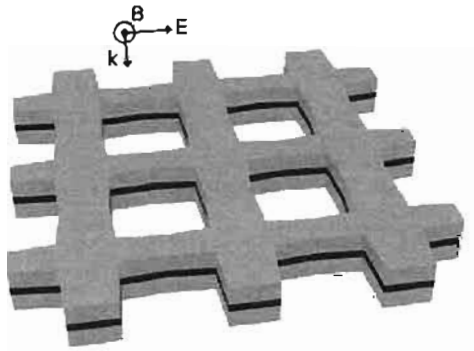
- Hologramas (óptico)
- Tinta color variable (óptico)
- Marcas de agua (óptico)
- Banda iridiscente (óptico)
- Hilo de seguridad (óptico)
- Impresión en relieve
- Microtexto (óptico)
- Exposición al UV (óptico)

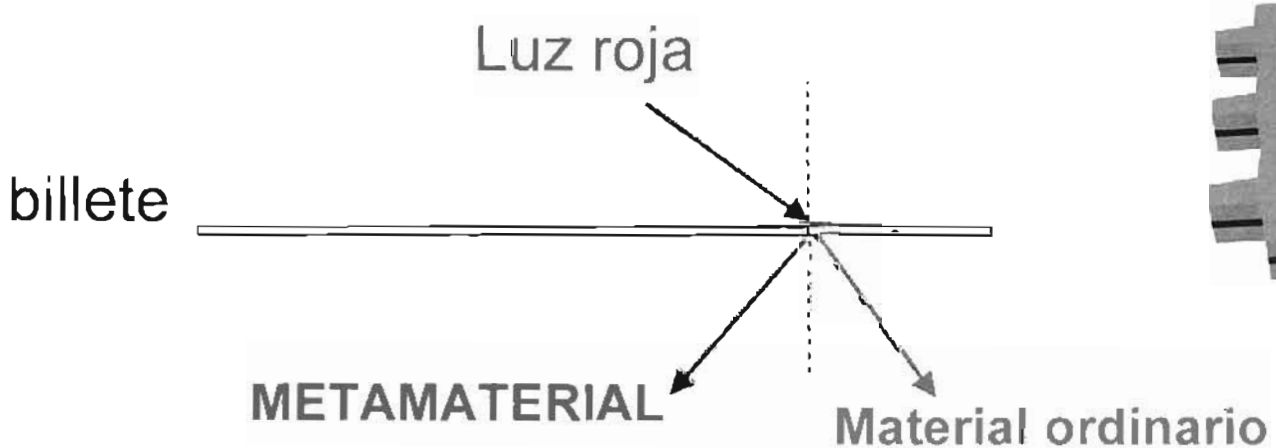


Seguridad óptica basada en MMs

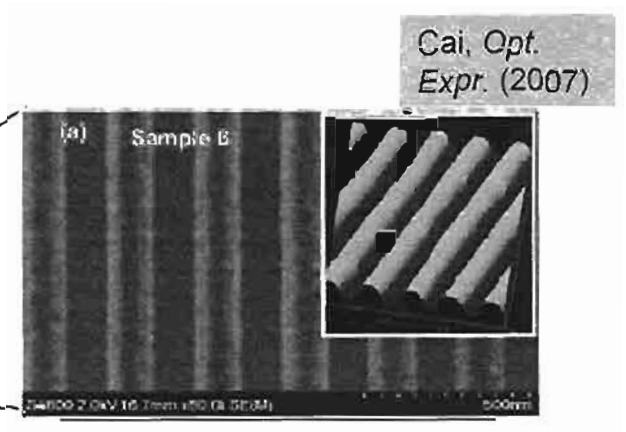


$n < 0$ para $\lambda = 780$ nm





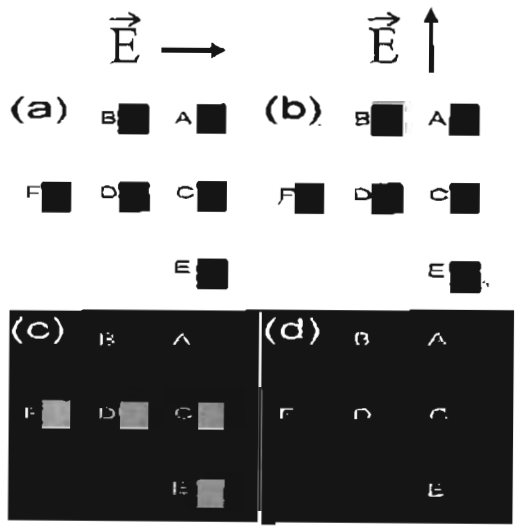
Seguridad óptica basada en MMs

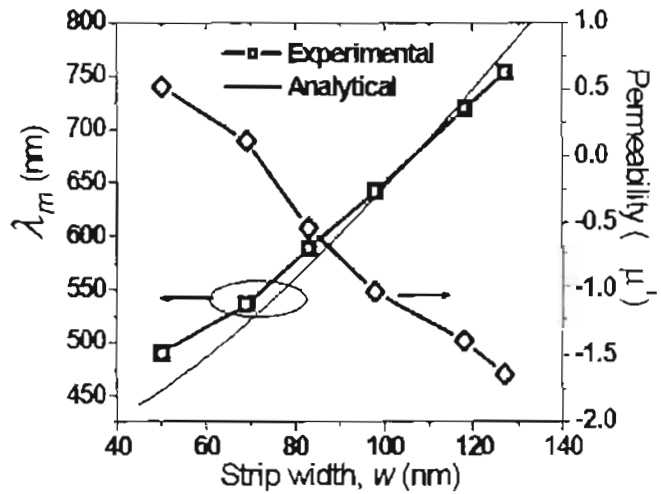


$\mu \neq 0$ para λ = visible

Transmisión

Reflexión





MMs para seguridad

- Respuesta óptica no convencional y de complejidad controlable ☺
- Visible → Posibilidad de inspección directa
- Visible → Nanotecnología → Equipos y procesos sofisticados: copia difícil ☺, baja reproducibilidad ☹
- Frecuencias ≤ THz → Inspección de segunda/tercera línea: se requiere instrumentación específica
- Frecuencias ≤ THz → Microtecnología → Equipos y procesos convencionales en microelectrónica: copia factible ☹, alta reproducibilidad ☺

Indice

Conclusiones

- MetaMaterial = estructura compuesta por sub-estructuras artificiales (meta-átomos), a las cuales debe sus propiedades.
- MM Electromagnético: la distancia entre meta-átomos es mucho menor que la longitud de onda de la radiación → Parámetros electromagnéticos homogéneos: $\varepsilon_{ef}, \mu_{ef}$
- Propiedades electromagnéticas exóticas (magnetismo óptico, índice de refracción negativo, invisibilidad, …) y "a voluntad" → gran flexibilidad en el diseño
- Aplicaciones en el visible → Nanofabricación
- Campo científico-técnico muy reciente → mucho por hacer

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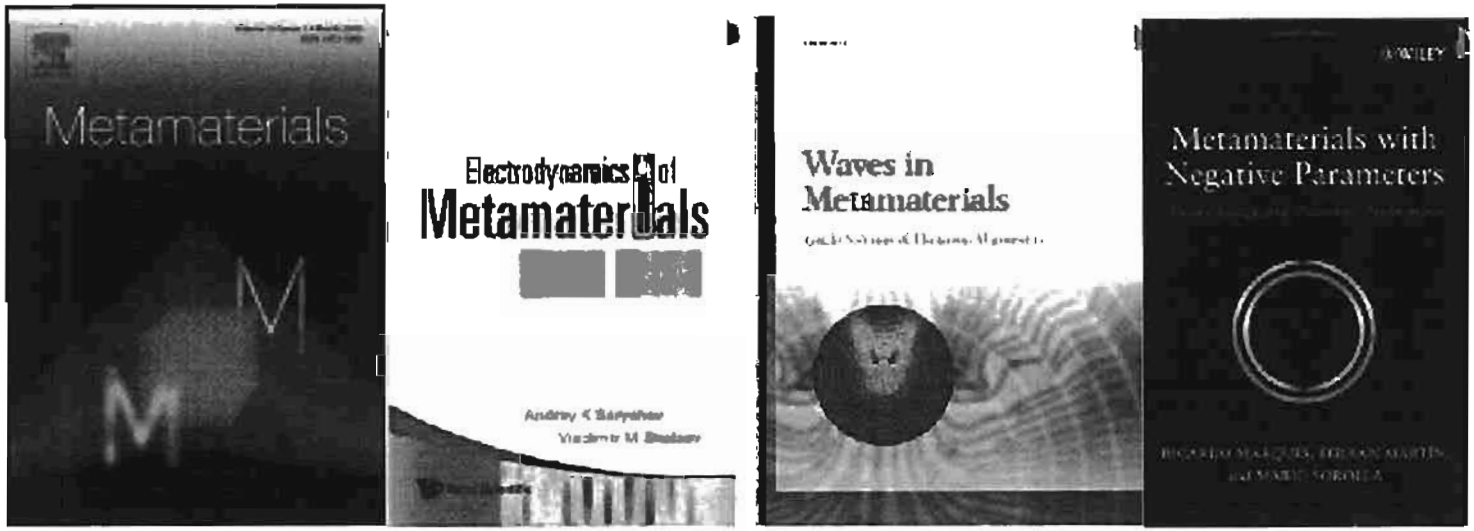

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Top Curr Chem (2005) 249: 317–349
DOI 10.1007/b104333

Imprinted Polymers

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1 Introduction

Recent progress in the area of host-guest chemistry has resulted in low molecular weight hosts capable of selective and strong complexation of a variety of guests in various matrix environments [1–3]. One drawback of such receptors is the difficulty in engineering them in useful formats for the recognition of guests of higher complexity or of larger size. Thus, general recognition strategies directed towards more complex targets based on artificial receptors remain an important challenge [4]. In this context the concept of molecular imprinting appears very appealing [5–7]. Here, monomers are chosen in order to complement functional groups of a template molecule. After incorporation of the monomer–template complexes in a cross-linked polymer matrix and removal of the template, binding sites remain that are capable of rebinding the template with high affinity and selectivity (Fig. 1).

The advantage of this "top-down" approach in receptor design lies in its use of the self-assembly principle to guide the binding groups to their positions in the receptor site; thus, the structure of the final binding site is, a priori, unknown. Recent advances in molecular imprinting have opened up new ways

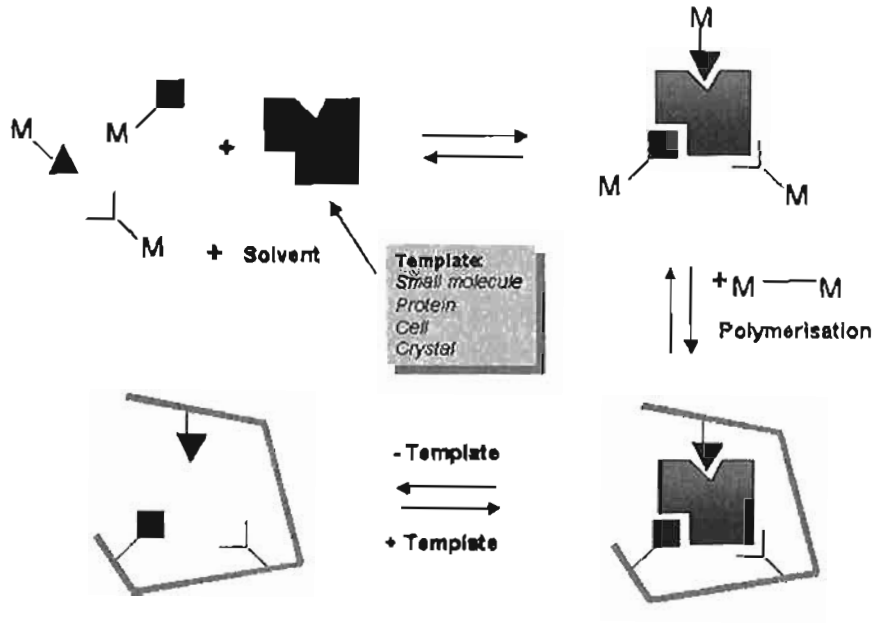


Fig. 1 General principle for imprinting of polymers starting from templates and monomers

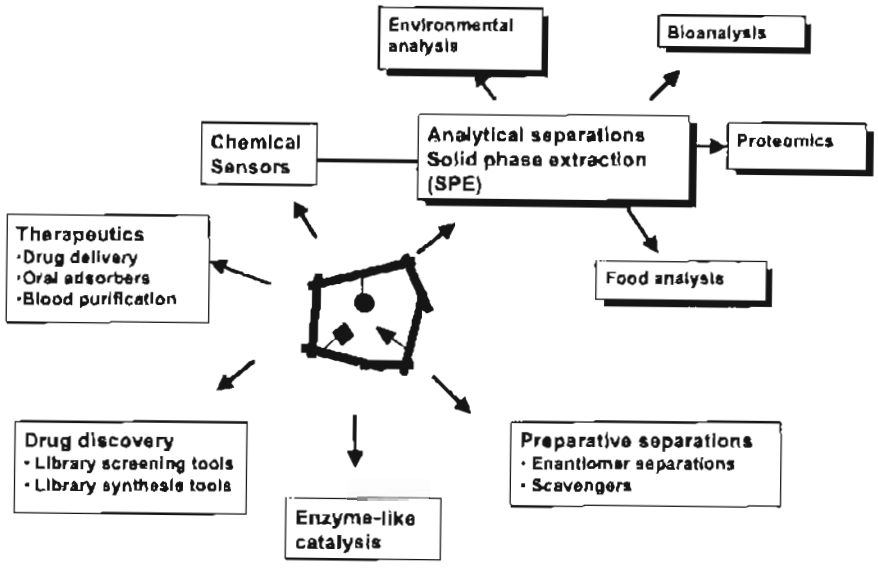


Fig. 2 Scheme outlining the main applications envisaged for MIPs

to custom-make robust molecular recognition elements with relatively little effort. A range of applications using these so-called molecularly imprinted polymers (MIPs) are under investigation (Fig. 2).

For instance, stable recognition elements capable of strong and selective binding of molecules could be used in areas with urgent needs for methods enabling selective separation [8], extraction [9] and sensing [10] of low molecular weight or macromolecular targets in biological fluids. Alternatively, such recognition elements could be used as scavengers to remove undesirable compounds from foods or biological fluids [11], for targeted delivery of drugs [12], or as tools in drug discovery [13]. If these recognition elements can be designed to bind specific proteins, a number of important applications in areas such as biotechnology (including downstream processing, sensors and diagnostics) can be foreseen.

Although MIPs are good at binding specific targets they have been less successful in catalysing chemical reactions [14]. However, using stable transition state analogues as templates, recent advances indicate that they may someday compete with their biological counterparts.

We will review here the recent advances in this field from the above-mentioned aspects. In the context of templates we will discuss the different roles templates have in molecular imprinting, from achieving molecular recognition to achieving catalysis, and the chemistry involved in achieving this using mainly the non-covalent imprinting approach. For a comprehensive coverage of the field, the reader is referred to a number of excellent books and reviews [5, 7, 10, 14–16].

2 General Approaches

MIPs are highly reticulated network polymers consisting of a common matrix structure and binding sites formed by a template present during polymer synthesis (Fig. 1).

The 3-D arrangement of the binding functional groups in MIPs is obtained by linking the functional monomers covalently or noncovalently to the template during polymerisation (Fig. 3). Removal of the template from the formed polymer then generates a structure complementary to the template structure. These sites can be reoccupied by the template or an analogous structure through reformation of the binding interactions present during synthesis or, alternatively, through weaker, kinetically more favourable interactions.

Essentially, three main approaches exist to date to generate high fidelity imprinted sites, which are distinguished by the nature of the linkage during synthesis and during rebinding.

The first example of molecular imprinting of organic network polymers introduced by Wulff was based on a covalent attachment strategy, i.e. covalent monomer–template, covalent polymer–template (Fig. 3A) [17, 18]. This approach has the advantage of a known stoichiometry between the functional

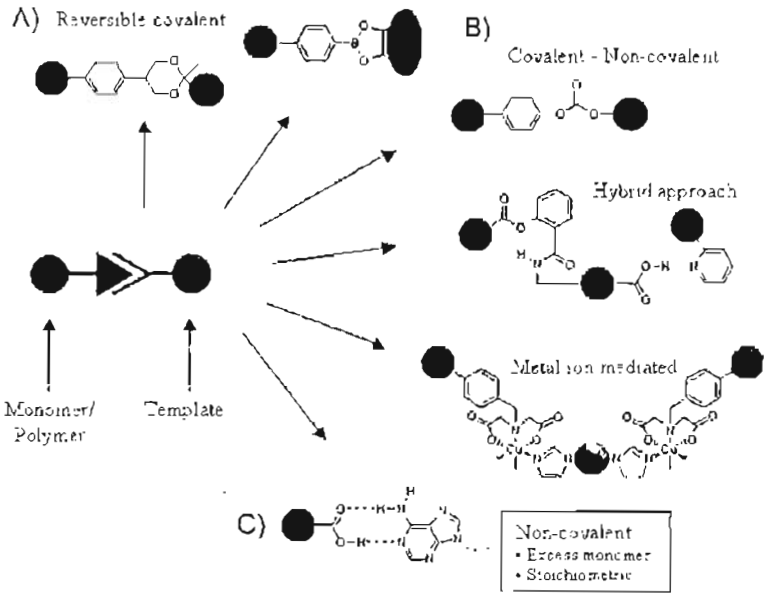


Fig. 3 Strategies used to place binding or catalytic functional groups at defined positions in imprinted sites of network polymers

monomer and the template. Provided that the template can be recovered in high yields, a high density of well-defined sites can be expected. One problem with this approach is the limited number of covalent linkages that satisfy these criteria. Furthermore, considerable synthetic effort may be required to prepare the template, and slow kinetics are often observed for rebinding by reformation of the covalent bond. This approach is therefore difficult to combine with applications where fast on-off kinetics are required. In this respect, the use of sacrificial spacers has found more widespread use [11]. Here the functional monomer is bound to the template through a disposable spacer that is removed after polymerisation is completed. This results in a disposition of the functional groups allowing rebinding to occur through hydrogen bonding interactions (Fig. 3B). Therefore, this approach can be more amenable to chromatographic applications and furthermore allows more freedom in the choice of polymerisation conditions (vide infra). However, the most widely used approach in imprinting involves functional monomers that are chosen to associate non-covalently with the template (Fig. 3C) [19]. Here the template is directly mixed with one or several functional monomers followed by polymerisation. It can thereafter be easily extracted from the polymer and recycled. Generally, the resulting materials can be directly used to perform separations with high affinity and selectivity, for instance as chromatographic stationary phases.

For example, a simple commodity monomer such as methacrylic acid (MAA) can be used to create good binding sites for a large variety of template structures

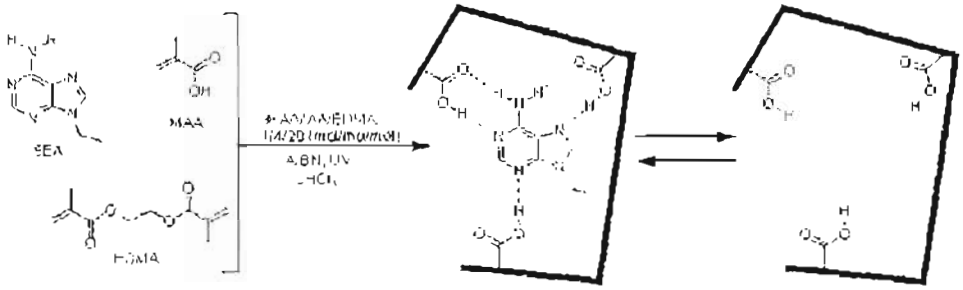


Fig. 4 Non-covalent imprinting of 9-ethyladenine (9EA) leading to highly cross-linked monoliths from which particles are obtained by repetitive crushing and sieving cycles

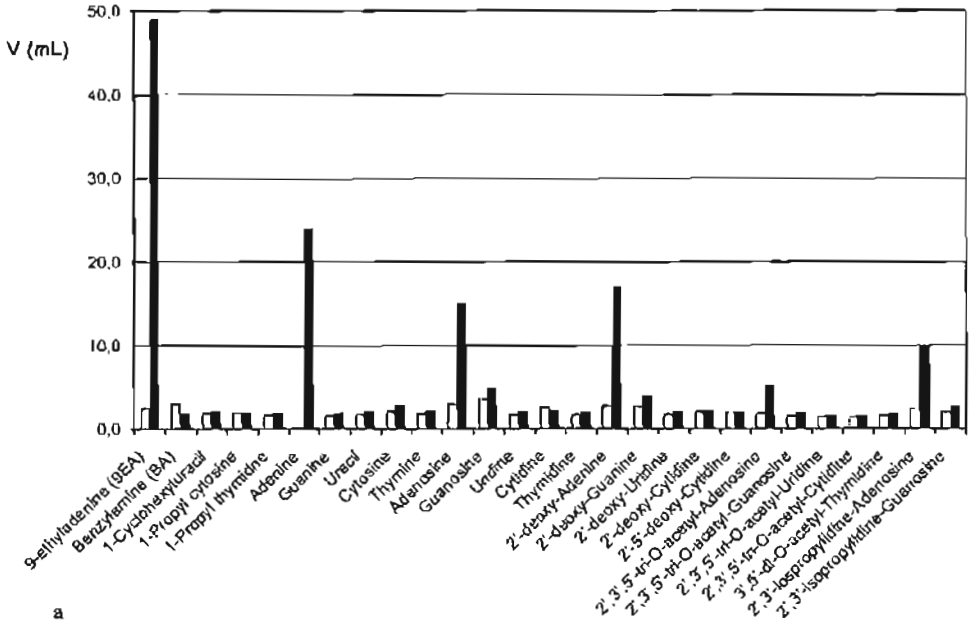
containing hydrogen bond- or proton-accepting functional groups (see Fig. 4 for the imprinting of 9-ethyladenine 9EA) [20]. MAA forms complementary hydrogen bonds or hydrogen-bonded ion pairs with the template, with individual binding constants ranging from units for weak hydrogen bonds to several hundreds for cyclic hydrogen bonds or hydrogen-bonded ion pairs formed in weakly polar aprotic solvents such as chloroform.

Otherwise, templates containing acids are often well targeted using basic functional monomers such as vinylpyridine, or amide monomers such as methacrylamide. For templates with multiple functionalities, obtaining the best result may require the use of a multitude of the above functional monomers. Often, the optimum combination is only found after time-consuming trial and error, which has spurred the development of parallel synthesis and assessment techniques and computational approaches to identify suitable functional monomers [21].

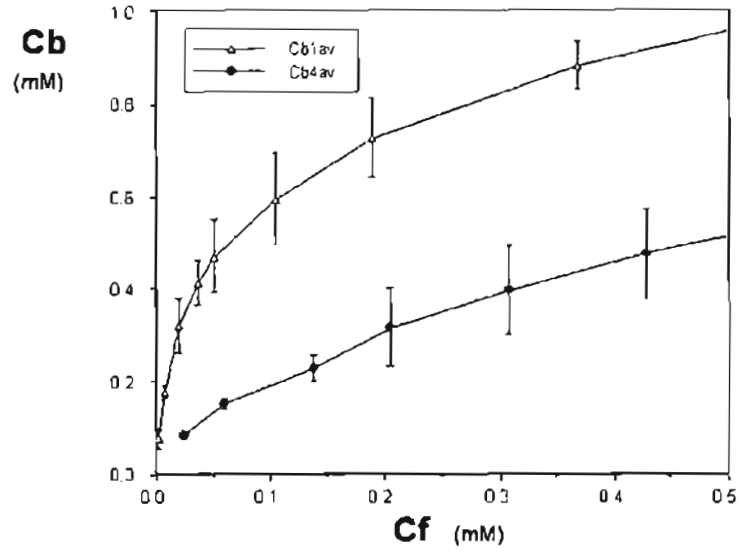
Although the preparation of a MIP by this method is technically simple it relies on the success of the stabilisation of the relatively weak interactions between the template and the functional monomers. This typically requires the use of solvents of low polarity and the addition of an excess of functional monomer (typically four equivalents, but sometimes higher) in order to ensure that the template molecule is complexed to a maximal degree. This, in turn, means that a large proportion of the functional monomer is not involved in complexation of the template and is instead distributed randomly throughout the polymer matrix during the polymerisation. This is a major cause of the high levels of non-specific binding and binding site heterogeneity observed in these materials.

The result is often a material that exhibits a small class of high affinity binding sites capable of discriminating the template from close structural analogues (see Fig. 5a) superimposed on a larger class of non-discriminating sites [20].

As shown for a polymer imprinted with 9-ethyladenine, these materials exhibit binding isotherms (Fig. 5b), that can only be fitted with multiple site models. This typically results in a poor performance when the materials are used a chromatographic stationary phases. Furthermore, it results in a low saturation capacity, which restricts the use of such materials mainly to low load



a



b

Fig. 5 a Elution volumes of the DNA bases, nucleosides and their derivatives from chromatographic runs using columns packed with a polymer imprinted with 9EA (*black bars*) or a control polymer imprinted with benzylamine (*white bars*). The mobile phase was acetonitrile/acetic acid/water: 92.5/5/2.5 (v/v/v). b Binding isotherms obtained from equilibrium binding experiments in chloroform showing the binding to a 9EA-imprinted polymer (*upper curve*) and a control polymer imprinted with benzylamine (*lower curve*). From [20]

analytical applications. One solution to this problem is to extend the monomer tool box drawing inspiration from the area of host-guest chemistry. Thus, designed functional monomers and recent examples of their use to achieve molecular recognition or catalysis are the subjects of the following sections.

3
Binding Site Design in Non-Covalent Imprinting

In the previous section, the various methods of molecular imprinting have been described. In this section we will focus on some of the more successful approaches that have been investigated towards the design of improved binding sites within MIPs. These concern firstly developments in "non-covalent" imprinting, especially with regards to the design of new "breeds" of functional monomers capable of stronger interactions than those traditionally used in imprinting and, secondly, on developments in "metal-mediated" imprinting.

The use of designed functional monomers also allows the opportunity to build in secondary functions, such as units capable of signalling a binding event or cross-linking functional monomers which can increase binding site fidelity. Developments of this nature will also be discussed.

3.1
New Host Monomers for Non-Covalent Imprinting

There have been a number of advances in recent years in the design of new functional monomers for non-covalent imprinting. Here, the aim is the preparation of monomers capable of strong binding to the template molecule, such that no excess of functional monomer is required during the imprinting process. The achievement of this ultimate goal has thus far been demonstrated in only a limited number of examples. To describe such examples Wulff has coined the phrase "stoichiometric non-covalent imprinting" [6].

One of the first reports of a designed functional monomer in molecular imprinting came from Takeuchi et al., [22] who used the bis-amidopyridine monomer 1 in the imprinting of barbital. The monomer presents a donor–acceptor–donor (DAD) array of hydrogen bond sites, which is complementary to the ADA sites within the template. The polymeric binding site obtained was postulated to resemble the structure of small molecule receptors prepared by Hamilton et al. [23] for the same purpose (Fig. 6). MIPs prepared with this monomer showed relatively high imprinting factors and a degree of selectivity for barbital over differently substituted barbiturates when tested in the chromatographic mode. Further, analytes where some of the hydrogen bonding sites had been removed were much less retained on these polymers. Takeuchi et al. extended their use of 1 to the imprinting of uracils (thymines) [24, 25].

Concurrently with this latter work, we used 1 in the imprinting of similar imide-containing templates, e.g. 1-substituted uracils [26, 27] and flavins (es-

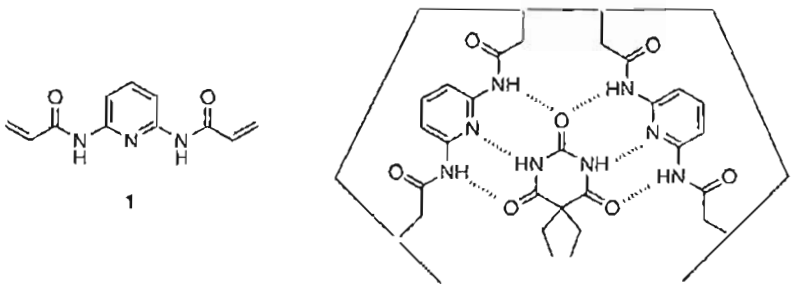


Fig. 6 2,6-Bis-acrylamidopyridine (1) and the proposed polymeric binding site with barbital

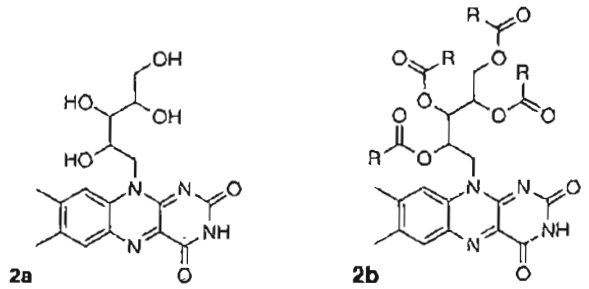


Fig. 7 Structures of 2a riboflavin and 2b template analogues used for imprinting (R=CH_3, C_2H_5, C_3H_7)

pecially riboflavin, vitamin B_2(**2a**)) (Fig. 7) [28]. We first quantified the solution binding of the monomer to the templates. By performing ^{1}H-NMR titrations (in $CDCl_3$), we obtained association constants of ca. 780 M^{-1} for the binding to (1-benzyl) uracil (**1BU**) and ca. 600 M^{-1} for a chloroform-soluble flavin derivative. This implies that, at "normal" imprinting concentrations (ca. 0.1 M in template), stoichiometric use of the monomer would lead to ca. 80% complexation of the template molecule. However, due to difficulties in solubilising 1 and **1BU** in the chosen polymerisation medium (containing EDMA as cross-linking monomer), we used much lower concentrations (ca. 0.05 M), while keeping the ratios of 1 and **1BU** stoichiometric with the template functionality. Even under these imprinting conditions, the obtained MIPs exhibited large imprinting factors.

We then extended our studies to the preparation of riboflavin-selective MIPs, again using 1 as the functional monomer. Due to the insolubility of riboflavin in organic media, we used a series of tetraesters (acetate, propionate and butyrate) as "template analogues" during the imprinting protocols and used 1 in a stoichiometric manner. The resulting MIPs showed extremely high imprinting factors (>100) for their respective templates when organic media where used as chromatographic mobile phases. Furthermore, recognition of riboflavin in predominantly aqueous phases (85% water) was also achieved.

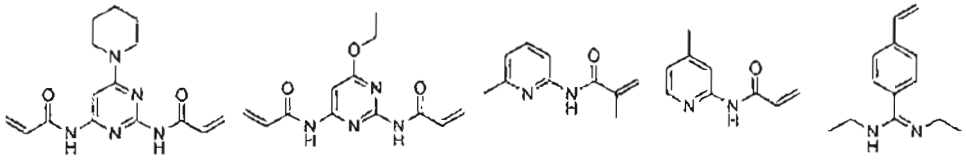
Aiming to increase the performance of our MIPs, we then prepared bis-amidopyrimidines 3 and 4 [27] (Fig. 8), expecting that the electron-donating substituents at the 6-position of the ring would lead to an enhancement of the hydrogen-bond accepting properties of the ring N3. However, the strengths of the binding to 1BU of both 3 (K_a ca. 600 M^{-1}) and 4 (K_a ca. 560 M^{-1}) were found to be lower than that with 1. This is in keeping with the reports of Sijbesma et al. [29], who reported that such molecules show a strong tendency to dimerise (K_d ca. 170 M^{-1} in $CDCl_3$), thus masking their binding affinity towards uracils. We estimate K_d(3) to be ca. 700 M^{-1}. Given that the K_a value for the 3:2 complexation is not so different from that obtained for 1:2 complexation, we believe that the intrinsic binding ability of 3 is higher than that of 1.

Despite this "masking" effect, anti-2 MIPs prepared with 3 as the functional monomer, under the same conditions used previously with 1, exhibited increased imprinting factors. A possible explanation is as follows: In the pre-polymerisation solution, 1 is either free or complexed (to 1BU). This leads to the formation of a mixture of non-specific and imprinted sites in the MIP. With 3, there will be a mixture of free 3, 1BU:3 complexes and $(3)_2$ dimers, which are duly incorporated into the polymer matrix. After extraction of 2, only monomer residues that were previously free or complexed to 2 are available for template rebinding as the dimers are now locked irreversibly into the 3-D network. Given the values for the association and dimerisation constants, it is reasonable to suggest that only limited amounts of 3 exist in the free state prior to polymerisation, thus reducing non-specific binding to the MIP.

In an interesting approach to imprinting in aqueous media, Komiyama et al. [30] have introduced the use of functionalised β-cyclodextrins as functional monomers in the imprinting of steroids and dipeptides, taking advantage of hydrophobic effects. In the latter example, it was demonstrated that the latent enantioselectivity exhibited by the host molecule was enhanced by the imprinting process.

Turning to the recognition of oxyacids, a number of reports concerning the use of novel monomers have appeared. In the majority of cases, these monomers offer only slightly improved binding compared to the commercially available functional monomers, with the exception of the amidine-based monomer of Wulff, which will be discussed later.

Steinke et al. [31] proposed the use of 2-amidopyridines for the imprinting of carboxylic acids, although no results on MIPs were presented. Whitcombe et al. prepared MIPs against glutamate-containing secondary metabolites of a fermentation process, using 6-methyl-2-(methacrylamido) pyridine (5) (Fig. 8) as functional monomer, with the aim of applying the materials to downstream processing applications. An association constant for the interaction of the monomer with acid (K_a ca. 100 M^{-1}) was measured prior to MIP preparation. The MIP prepared using 5 performed less well than another prepared using 4-aminostyrene as functional monomer and no further studies were performed with this MIP. We have also prepared a series of such monomers and found that subtle increases in solution association with model acids may be achieved via



3 4 5 6 7

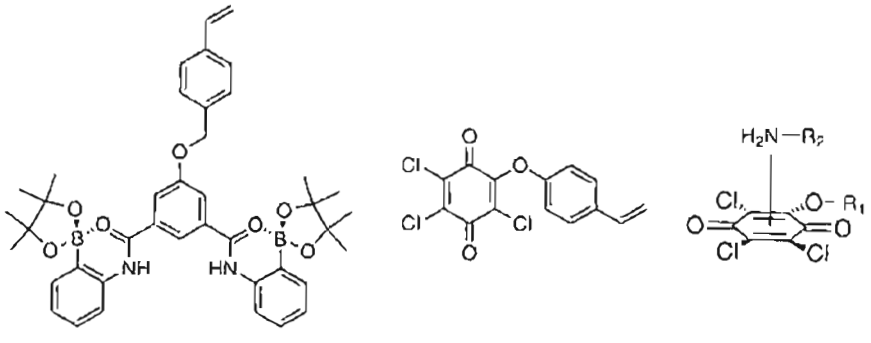
Fig. 8 Some monomers used for stoichiometric imprinting of imides (3, 4) carboxylic and phosphonic acids (5–7)

simple variation of substitution patterns (at the pyridine 4- and 6-positions). There appears to be a delicate balance between enhanced basicity and steric factors affecting the extent of association, with monomer **6** showing the optimum binding properties (K_a=780 M^{-1} in $CDCl_3$) [33].

Spivak and Shea prepared a range of functional monomers for the imprinting of acids [34]. Included were 2-amidopyridines, adenine-based monomers and monomers containing guanidinium functions. However, MIPs prepared from these monomers showed negligible imprinting effects. This is presumably due to the fact that the poor solubility of the monomers necessitated the use of an extremely polar solvent (DMF) as polymerisation solvent.

To date, the most successful functional monomer for the imprinting of neutral acids is the amidine-based monomer **7** reported by the group of Wulff [35]. This monomer is capable of engaging in electrostatic and cyclic hydrogen bonding interactions with carbon and phosphorous acids. In $CDCl_3$, the interactions (K_a>10^6 M^{-1}) are strong enough to allow beaded MIP preparation via traditional, aqueous-based suspension polymerisation techniques [36]. The association is also strong in CD_3CN (K_a≈10^4 M^{-1}). The stoichiometric use of **7** in such solvents leads to >95% complexation prior to the polymerisation, which has been shown to translate to a near-quantitative yield of imprinted sites in the final polymer. A limitation of **7** is that the association strength falls dramatically on moving to solvents of yet higher polarity, e.g. DMSO-d_6 (K_a<10 M^{-1}), due to the adoption of an unfavourable conformation for binding. The highly impressive use of **7** in the preparation of catalytically active MIPs will be discussed in more detail in the following section.

Whitcombe et al. described two novel functional monomers for the preparation of MIPs against ampicillin, an antibiotic (Fig. 9) [37]. To target the carboxylic acid (in its anionic form) they prepared a polymerisable version of a previously reported receptor (**8**). Unfortunately, the binding of this monomer to carboxylates (ca. 280 M^{-1} in DMSO-d_6) was an order of magnitude lower than the receptor upon which the monomer was based. It was suggested that the decrease in binding strength arose from the electron-releasing group by which the polymerisable function was introduced. To target the amino group the chloranil-based monomer **9** was prepared. According to Job plot analysis, one amino group should be complexed by two molecules of **9**. The interaction between the template and **9** (in DMSO-d_6) was too strong to



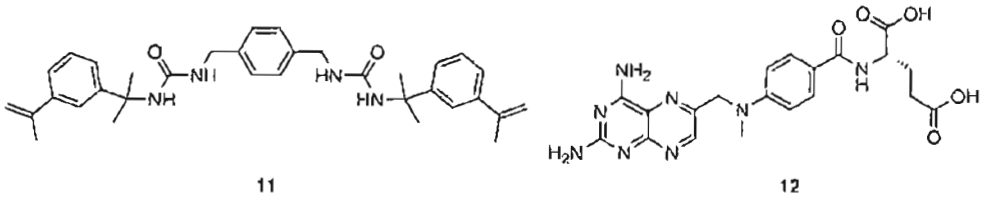
8 9 10

Fig. 9 Novel monomers used in the "orthogonal" imprinting of ampicilllin. 10 is the proposed complex formed between 9 and a 1° amine

be determined quantitatively (via ^{1}H-NMR titration), but was estimated to be $>10^4$ M^{-1}. Stoichiometric use of **9** (postulated to give a complex such as **10**), together with monomer **8**, led to a MIP capable of selective ampicillin uptake in buffered aqueous media. It is also interesting to note that the monomers may be used in an orthogonal manner, as there are no competing monomer–monomer interactions.

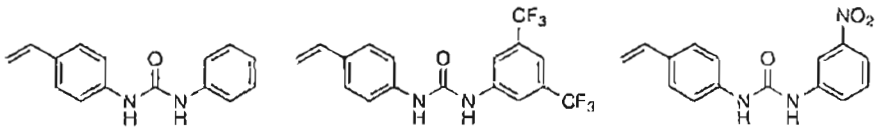
We have recently introduced a new type of monomers, containing 1,3-di-substituted urea moieties, for targeting oxyanions (Fig. 10) [38]. This moiety has been extensively used in small-molecule receptors [39] and it has been shown that, by manipulation of the urea substituents, extremely strong binding to oxyanions may be achieved [40], even in polar environments, e.g. DMSO.

In our first study we prepared the bis-urea monomer **11** and used it in an attempt to prepare MIPs able to recognise the anti-cancer drug methotrexate (**12**), which contains a glutamic acid residue. Thus, a MIP was prepared against the tetrabutylammonium (TBA) salt of N-*Z*-L-glutamic acid using **11** stoichiometrically. Prior to this we determined K_a between and **11** and bis-TBA-glutarate to be ca. 1,500 M^{-1} (DMSO-d_6). Initial chromatographic mode testing, using pure acetonitrile as the mobile phase, led to minimal differences being observed between the MIP and non-imprinted (NIP) control polymer with respect to the



11 12

Fig. 10 A bis-urea monomer, targeted towards glutamate recognition, and the anti-cancer drug methotrexate



13 14 15

Fig. 11 Bis-aryl mono-urea monomers providing for strong binding to oxyanions

retention of N-*Z*-L-Glu. However, addition of small amounts (1–2%) of a base, triethylamine, to the mobile phase caused large differences in the behaviours of the polymers. Thus, the "template" now exhibited far greater retention on the MIP. Further, the MIP was able to separate an equimolar mixture of N-*Z*-L-Glu, N-*Z*-L-aspartic acid and N-*Z*-glycine, while the NIP was not. Finally, the aim of the study was achieved by showing that the MIP was also capable of retaining 12.

We have since turned our attention to the preparation and use of polymerisable 1,3-diaryl mono-ureas (Fig. 11) [41, 42]. These monomers provide for strong interactions in polar media, which may be further tuned by the appropriate choice of substituents on the phenyl groups. A "base" value for this interaction strength is provided by 1-(4-vinylphenyl)-3-phenyl urea (13); complexation of 13 with benzoate (in DMSO-d_6) gives K_a ca. 1,300 M^{-1}.

Placement of electron-withdrawing groups (NO_2, CF_3, etc.) on the 3-phenyl ring lead to significant increases in binding, e.g. binding of 14 to benzoate gives K_a ca. 9,000 M^{-1}. We have used monomer 15 (K_a with benzoate ca. 8,000 M^{-1}) to create another MIP against *N*-*Z*-L-glutamic acid. Once again, base-modified mobile phases are necessary for the recognition properties of the MIP to become "activated". However, in comparison to the 11-based polymers, far greater binding strength is observed. Thus, addition of water (6%) to the mobile phase leads to elution of the template from the NIP, though not the MIP. At a water content of 7%, the template also elutes from the MIP, but is much more retained than on the NIP. In equilibrium binding experiments, a significant difference between the uptake of the imprinted enantiomer and its antipode was observed (the difference in uptake amounting to an impressive amount of ca. 13 μmol g^{-1} polymer).

3.2
Introducing Secondary Functions to Non-Covalent Binding Monomers

As well as enhancing binding strength by the design and synthesis of novel binding elements, there is also the possibility to introduce interesting secondary functions to the monomer, e.g. signalling subunits, cross-linking ability.

In this context, the preparation of monomers that can give a readable signal of the binding event within the polymer would surely advance the use of MIPs in sensory applications and some examples of non-covalent functional monomers possessing such properties have begun to appear.

The first example of this kind was reported by Turkewitch et al. [43] Imprinted polymers were prepared against cAMP incorporating a fluorescent dye, trans-4-[*p*-(*N*,*N*- dimethylamino)styryl]-*N*-vinylbenzylpyridinium chloride, as an integral part of the recognition cavity. This served as both the recognition element and the measuring element for the fluorescence detection of cAMP in aqueous media.

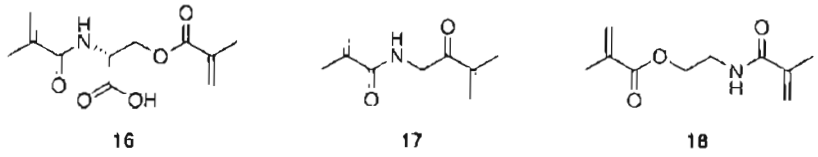
Another recent example of a monomer exhibiting both binding and signalling properties came from Takeuchi's group [44]. Imprinted polymers exhibiting selectivity for 9-ethyladenine were prepared by combining MAA and vinyl-substituted zinc(II) porphyrin as functional monomers (see also the following section). Compared to MIPs using only methacrylic acid or zinc porphyrin as a functional monomer, the terpolymer showed higher affinity and selectivity for the template. Interestingly, these polymers showed fluorescence quenching correlating with the binding of 9-ethyladenine, and the quenching was significant in the low-concentration range, suggesting that the high-affinity binding sites contain the porphyrin residue.

Monomer 1 has been shown, by ourselves [27] and Takeuchi [45], to be fluorescence active. In our work, addition of the template **IBU** to a chloroform solution of 1 leads to quenching of this fluorescence [33]. This is also carried through to the polymeric systems, i.e. the MIP and the control NIP, as seen from equilibrium binding experiments (although the emission maximum is shifted in the polymers, a phenomenon which is discussed below). The quenching of fluorescence agrees well with the quantity of IBU rebound to the polymers. Further, the quenching of fluorescence for the MIP is far greater than that seen for the NIP, again in agreement with the earlier chromatographic and rebinding experiments. Addition of non-template species leads to far less fluorescence quenching on the MIP, but only minimal alteration of the response of the NIP, thus showing the existence of selective sites within the MIP. Monomer 3 shows similar effects, but there is a more pronounced selectivity in the fluorescence quenching response of the MIP, again consistent with the earlier results obtained from chromatographic testing.

Conversely, Takeuchi et al. have recently reported that MIPs prepared against barbital using 1 as the functional monomer exhibit enhancements in their fluorescence emission when template binding occurs [45]. While interesting, the inherent fluorescence of the barbiturate molecule appears to have been overlooked.

The urea monomer 15 exhibits a chromogenic response to carboxylate binding in solution, with a bathochromic shift in the absorbance maximum being observed (from 349 nm to 364 nm) [41]. Although this shift is small, it is sufficient for the binding event to be seen with the naked eye. These effects are also carried through to the anti-N-*Z*-L-Glu MIP, which exhibits a stronger chromogenic response than the NIP. We are currently investigating the use of the urea moiety as a platform for the generation of binding monomers, which show larger responses (chromogenic or fluorogenic) on binding.

Other examples of non-covalent functional monomers combining both binding and reporting ability are rare. Shea et al. [46] have reported some



16 17 18

Fig. 12 Novel cross-linking functional monomers by Spivak et al.

interesting examples recently, but their use in MIPs has yet to be demonstrated.

Monomers 1 and 3 are also, potentially, cross-linking monomers. That they are incorporated into the polymeric matrix via polymerisation of both their C=C bonds is indicated by the change in the fluorescence emission maximum of the monomer units once polymerised; the polymers showing emission maxima very similar to those of saturated models [27]. These measurements show, at least qualitatively, that "double" incorporation of 1 and 3 into the polymer matrix occurs. This is probably an added factor in their success as binding monomers in molecular imprinting.

Most of a MIP is made up from cross-linking monomer. More recently, in recognition of this fact, Spivak et al. have introduced a series of small-molecule cross-linkers containing carboxylic acid or amide residues for use in imprinting protocols (Fig. 12) [47–49].

The results of imprinting using such cross-linking monomers, compared to more "traditional" protocols, have been reasonably impressive. The use of 16 leads to enhancements (cf. using methacrylic acid as functional monomer) in enantioselectivity when imprinting nicotine and chiral amines [47]. Further, MIPs prepared using 17 showed much better enantioseparation of N-protected amino acids than those formulated with the more traditional cross-linker, EDMA [48]. Most recently, this group has coined the term "OMNiMIP" (One MoNomer MIPs) after the discovery that monomer 18 could be used alone (i.e. in the absence of other functional or cross-linking monomers) to create MIPs showing good enantioselectivity, albeit for a limited range of target species [49]. Also, as these monomers function via weak interactions, the problems relating to binding site heterogeneity and the need for low polar media during imprinting are not overcome using this approach.

3.3 Binding Site Monomers in Metal-Mediated Imprinting

The use of functional monomer–metal ion–template complexes in imprinting protocols is rare, which is surprising given that metal ion–ligand complexes are generally extremely strong, even in water. One problem perhaps lies in the preparation of well-defined ternary (or higher) metal ion complexes. Indeed, there are few examples where the structures proposed to be present in the imprinting mixture have been definitively confirmed, e.g. by crystal structure determination.

Notable early examples of this technique came from the group of Arnold, particularly the use of polymerisable Cu(II)-iminodiacetic acid complexes for the imprinting of benzimidazole-containing molecules [50]. More recently, Striegler has used the Cu(II) complex of a polymerisable ethylene triamine ligand as a functional monomer for the imprinting of carbohydrates (mono- and disaccharides) with some success [51–53]. However, the use of Cu(II) complexes can lead to problems in free radical polymerisation protocols, especially with regards to incomplete polymerisation of monomers.

To avoid such problems, the group of Shea used polymerisable Ni(II)-nitrilotriacetic acid (NTA) complexes for the preparation of MIPs capable of recognition of histidine residues in small oligopeptides [54, 55]. Histidine is known to form an octahedral ternary complex of considerable strength with Ni(II) and NTA in water (K_d=0.0093 mM). The 1:1:1 ternary template complex (19) between His-Ala, Ni(II) and the polymerisable NTA ligand (20) is depicted in Fig. 13.

This ternary complex was characterised by absorption spectroscopy and mass spectroscopy. It is notable that both the imprinting step and the subsequent rebinding experiments were performed in purely aqueous media. In the rebinding step, uptake of His-Ala was found to exceed that of His-Phe, while minimal binding of Ala-Phe was observed. This implies firstly that there is restricted access to the binding cavity in the case of the larger dipeptide and, secondly, that the terminal His-residue is required for binding. On imprinting His-Phe, no selectivity for the two dipeptides was observed, indicating accessibility to the binding site for the smaller dipeptide. Further, when a pentapeptide, His-Ala-Ala-Ala-Ala, was imprinted, the uptake of both His-Ala and His-Phe by the MIP was found to be greater than that of the template (and His-Ala-Phe and His-Phe-Ala-Ala-Ala). Finally, kinetic binding studies, using His-Trp as a fluorescent probe, indicated that the rebinding was reasonably fast, leading to equilibration in ca. 1 h.

As mentioned in the previous section, Takeuchi's group used a polymerisable Zn(II)-porphyrin complex, in combination with methacrylic acid, for the preparation of MIPs against 9-ethyl adenine [44] and (–)cinchonidine [56]. The

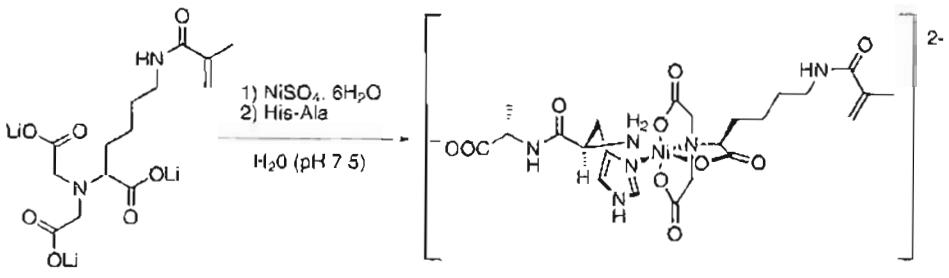
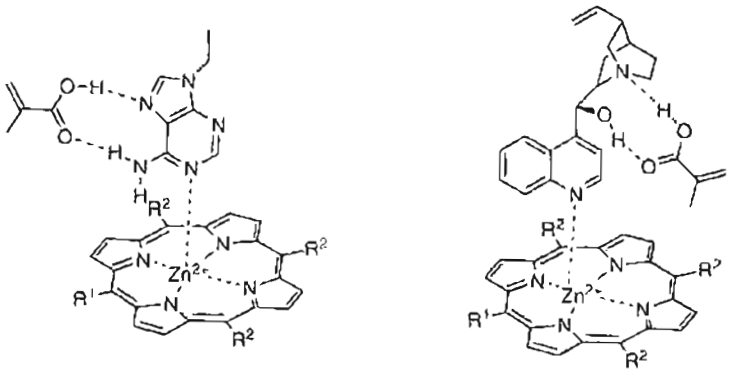


Fig. 13 Formation of template complex in the imprinting of His-containing peptides



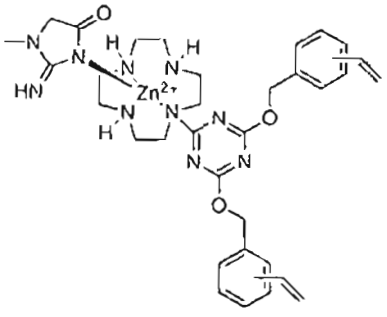
21 22

Fig. 14 Proposed complexes in the imprinting of 9-ethyladenine and (–)-cinchonidine using a polymerisable Zn(II)-porphyrin functional monomer possessing secondary signalling properties in combination with methacrylic acid. $R^1 = CH_2{=}C(CH_3)COOC_6H_4$; $R^2 = (CH_3)_2CHC_6H_4$.

proposed complexes, **21** and **22**, are shown in Fig. 14. There appears to be a high degree of co-operativity in each of these systems, as MIPs made with each monomer individually were shown to be less effective in uptake of the template. In the case of (–)-conchinidine MIP, the uptake of (+)-cinchonine was lower than that of the template, indicating a certain degree of diasteroselectivity. In both systems, it was demonstrated that rebinding of the template was accompanied by a change in UV-Vis and/or fluorescence behaviour, while the response was much lower for the non-template species tested. Thus, a secondary signalling property is introduced by incorporation of the metal ion complex into the binding cavities of the polymers. However, it should be mentioned that very little characterisation data for the proposed complexes was provided. Further, the polymerisation was performed in chloroform and rebinding studies were carried out predominantly in dichloromethane. It would be interesting to see if the proposed ternary complexes could be of use for imprinting in more polar media.

König et al. have recently published on the use of a polymerisable Zn(II)-cyclen complex in the imprinting of creatinine; the template complex **23** is depicted in Fig. 15 [57].

This report is one of the few in which the structure of the imprinted complex has been definitively confirmed (by X-ray crystallography). Also notable is that imprinting was carried out using water as the polymerisation solvent and that the rebinding experiments were conducted in water at physiological pH. In solution, the Zn(II)-cyclen complex is known to bind to thymine in preference to creatinine, with the association constant being ca. 34 times higher. Imprinting of the complex led to a MIP capable of reversing this preference, with creatinine absorbed ca. 3.5 times more than thymine. Further, the MIP



23

Fig. 15 Ternary template complex used in the imprinting of creatinine

could be used in repeated absorption-desorption cycles with little loss in activity. Control polymers lacking the metal ion displayed no significant affinity for either creatinine or thymine, while a flavin absorbed non-specifically to all polymers.

A further recent example has come from the group of Wulff [58, 59]. This may be viewed as a combination of non-covalent and metal-mediated imprinting approaches. Thus, a functional monomer possessing both an amidine group (for acid binding) and a Zn(II)-aza-macrocycle complex, separated by a suitable spacer, has been used in the preparation of catalytically active MIPs, as will be discussed later.

3.4
Molecularly Imprinted Dendrimers

Very recently, in a new approach to binding site design, Zimmerman et al. have introduced the idea of imprinting in dendrimers, whereby one macromolecule is furnished with a single binding site (similar to the case of enzymes) [60]. While the synthetic effort is certainly greater than in traditional imprinting protocols, there is the benefit that homogeneous binding sites may be formed using this technique. Developments in this work have been reviewed recently [61].

4
Catalysis with Imprinted Polymers

Given the receptor-like molecular recognition properties displayed by several imprinted polymers, the idea of combining the recognition event with a chemical transformation in an enzyme-like fashion seems obvious. This approach to heterogeneous catalysts was first proposed by Wulff in the early 1970s, but only the last couple of years has seen promising advances towards this end [14].

Enzymes catalyse a large variety of chemical and biochemical reactions with high reaction rates and specificity under relatively mild conditions. Thus, chemists have sought to create mimics that could match the catalytic properties of enzymes. Early approaches to enzyme mimics were based on macromolecular receptors, e.g. cyclodextrins or crown ethers containing suitably placed functional groups to mimic the amino acid residues known to be involved in the catalytic mechanism [62]. Such models were able to mimic the main features of enzymes (i.e. substrate selectivity, Michaelis-Menten kinetics and turnover) although commonly showing modest rate enhancements using activated non-natural substrates and non-aqueous environments. However, no such simple model system shows catalytic activity on a par with the biological counterparts.

Catalytic antibodies have come closer in this regard and have been regarded as the most successful enzyme mimics [63, 64]. Here, antibodies are elicited using antigens containing stable transition state analogues (TSAs), for the reaction to be catalysed, as haptens. These mimic the shape and charge of the transition state of the reaction to be catalysed and define the active site of the antibody. This builds on the idea of Pauling [65] and Jencks [66] that enzymes owe their enormous power to catalyse a reaction mainly to their ability to lower the energy required for passing the transition state of the reaction.

Based on similar concepts, MIPs showing catalytic activity have been developed. Here, first of all, a proper cavity equipped with catalytically active functional groups is required. This should furthermore exhibit binding and shape complementarity towards the substrate or the transition state of the reaction.

There are two main strategies to achieve this (Fig. 16). The most common method is to place suitable functional groups in the cavity by choosing the substrate or the product of the reaction, or alternatively analogues of these, as the template. In line with catalytic antibodies, however, the most successful approach is the use of stable transition state analogues (TSA) as templates.

The second strategy is to incorporate a low molecular weight metallorganic catalyst as a complex with a template molecule (e.g. the substrate, its analogue or a transition state analogue) to form the active sites in the polymer. In this

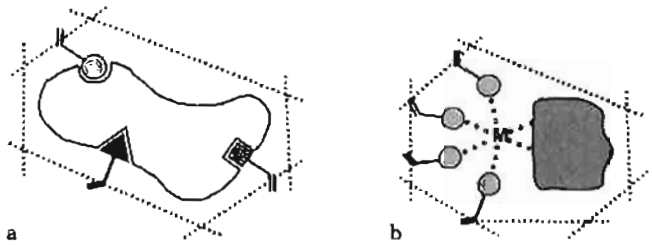


Fig. 16 Two approaches to achieve molecularly imprinted catalysis (MIC). a Bio-inspired MICs by constructing catalytically active sites using stable transition state analogues. b Chemo-inspired MICs by imprinting of a metallorganic catalyst in a complex with, e.g. a transition state or substrate analogue

case the polymer plays the role of improving an already established solution catalyst. Thus, apart from achieving simple immobilisation of the catalyst, the imprinted site may allow the introduction of further catalytically active or binding functional groups and to provide shape selectivity in the reaction. In this manner the selectivity and activity may be improved in the resulting heterogenous catalyst.

These two main strategies for creating MICs will be described in more detail below. Under the first group we will focus on models inspired by hydrolytic enzymes. These constitute by far the most widely studied and developed group and also the group that has come closest in mimicking the key features of enzymes. As stated in the introduction we have limited the review to include systems based on organic network polymers. Comprehensive reviews of earlier work can be found elsewhere [14, 67].

4.1
Bio-inspired MIPs Catalysing Hydrolysis Reactions

In the chemistry of enzyme mimics one general strategy is to generate a host that is capable of binding to a transition state analogue (TSA) of a reaction. Upon removal of the template the host should behave as an artificial enzyme for the chosen reaction [68]. This strategy has met with considerable success in the field of catalytic antibodies [64], which further inspired research to produce imprinted catalysts based on the same principle. The first examples of catalytically active MIPs were published in the 1980s. Thus templates similar or identical to previously used antibody haptens were used to create an imprinted polymer [14]. The functional monomers in these systems have to fulfil several requirements that are somewhat oppositional. On the one hand, the functional monomers have to form a stable complex with the template molecule during the polymerisation step, leading to their incorporation into the polymer matrix in the correct orientation and position. On the other hand, the interactions must be readily reversible in order to allow release of the template to form the empty cavity. Further, during the catalytic process these groups must bind the substrate and/or the transition state and provide catalysis allowing for fast kinetics and product release.

The earliest and most extensive efforts towards MIP-based catalysts concerned the mimicking of serine protease enzymes. This is mainly due to the extensive knowledge available concerning their mechanism of action and the structure of the active site and intermediates [69]. A further factor is the fact that serine proteases, lipases, cholesterases and other hydrolytic enzymes share similar catalytic machineries and mechanisms. Chymotrypsin, an enzyme with a well documented catalytic mechanism, has been the model of choice in these efforts [68].

Most reports up to now have concerned imprinted polymers that mimic their hydrolytic activity by incorporating some or all of the key features of the active site, e.g. the Ser-His-Asp catalytic triad, transition state stabilisation and a stereoselective binding pocket, into the synthetic polymer.

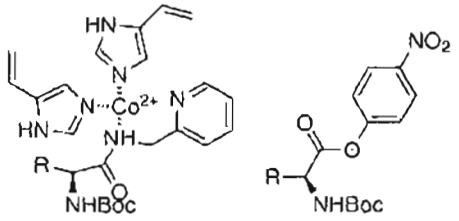


Fig. 17 Template complex and substrate in esterase mimic by Leonhardt and Mosbach

In the first report by Leonhardt and Mosbach, imidazole residues were employed as the catalytically active groups to hydrolyse activated amino acid nitrophenyl esters (Fig. 17) [70]. A complex comprising 2-vinyl imidazole and a substrate analogue coordinated to Co(II) was copolymerised with DVB. The MIPs showed a four- to eightfold rate enhancement of the hydrolysis of BOC-Leu(or Met)-*p*-nitrophenylester over the control polymer containing statistically distributed imidazole groups.

With the hope of creating more active catalysts, the use of stable TSAs as templates came soon after the first reports on catalytic antibodies. By using a phosphonic acid as a TSA template for the hydrolysis of 4-nitrophenyl esters, polymer catalysts developed by the groups of Mosbach [71] and Ohkubo [72] showed modest rate enhancement (ca. sevenfold for Ohkubo's catalyst) with reference to the uncatalysed solution reaction. The lack of proper controls in some of these reports makes it difficult to ascribe these effects to the presence of templated sites alone.

More recently Ohkubo and co-workers synthesised polymers imprinted with a racemic TSA corresponding to the hydrolysis of *Z*-Leu-4-nitrophenyl ester using *N*-acryloyl-L-histidine methylester as the functional monomer (Fig. 18) [73, 74]. MIPs were synthesised using different cross-linkers and by using styrene as a hydrophobic co-monomer. Thus polymers were obtained giving faster hydrolysis for the L-isomer over the D-isomer by factors of 1.15–2.54 and showed catalytic rate enhancements of 3.4–29, depending on the cross-linkers and co-monomers used (see Table 1).

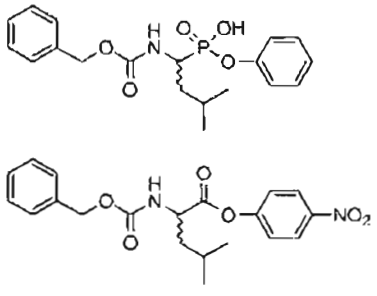


Fig. 18 *rac*-TSA and substrate used by Ohkubo et al.

Table 1 Comparison of kinetic data in reports on MIPs exhibiting esterase-like activity

Ref.	Template type	Template binding	Polymer format	Substrate type	k_{cat}/k_{uncat}	Enantio-selectivity	k_{cat}/k_{cat}	K_M (mM)	k_{cat} (min^{-1})	v_{max} (mM/min)	K_i (mM)
[70]	SubA	I	CM	PNP	–		4–8				
[71]	TSA	I	CM	PNP	6.7		-				
[74]	TSA	II	CM	PNP	29[a] 3.4[b]	1.15[a] 2.54[b]	-				
[75]	TSA	II+III	CM	PNP (ethyl ester)	10	1.85	2.5	5.4	0.0014	-	-
[35]	TSA	IV	CM	Phenyl ester	102–235		5.0	0.6	0.00008	-	0.025
[36]	TSA	IV	CM	Carbonate	588		7.8	5.01	0.012	0.023	0.094
[36]	TSA	IV	CM	Carbamate	1435		4.2	3.33	0.022	-	0.285
[78]	TSA	IV	Beads	Carbonate	168[c] 293[d]		24[c] 9.7[d]	13.4[c]	0.004[c]	0.008[c]	0.22[c]
[78]	TSA	IV	Beads	Carbamate	140[c]		11[c]				
[80]	TSA	IV	CM	Cholesterol carbonate	27		2.4	3.7	0.000222	-	0.9
[79]	TSA	IV	CM	Phenyl ester	325	1.39[f] 1.65[g]	79	0.51	0.000474	-	0.016

Table 1 (continued)

Ref.	Template type	Template binding	Polymer format	Substrate type	k_{cat}/k_{sol}	Enantio-selectivity	k_{cat}/k_{ctrl}	K_M (mM)	k_{cat} (min^{-1})	v_{max} (mM/min)	K_I (mM)
[81]	SubA	I	Without emulsion	PNP	1.8		-	1.63	-	0.0625	-
[58]	TSA	I+IV	CM	Carbonate	3264[h]		61.5[h]	2.01[h]	0.035[h]	-	-
					76570[i]		80.1[i]	0.58[i]	28.0[i]		
[82]	TSA	IV	Microgels	Carbonate	530		-	2.38	-	0.0804	-

k_{cat}/k_{sol} is the enhancement determined from pseudo-first-order kinetics as the ratio between the first-order rate constants of the imprinted polymer(k_{cat}) and the solution (k_{sol}). k_{cat}/k_{ctrl} is the imprinting efficiency determined from the first-order rate constants of the imprinted polymer (k_{cat}) and the control polymer t (k_{ctrl})

SubA substrate analogue, *TSA* transition state analogue, *PNP* *p*-nitrophenyl ester, *I* metal coordination, *II* non-covalent, *III* covalent, *IV* stoichiometric non-covalent, *CM* crushed monolith.

[a] Cross-linker: ethylene bisacrylamide.

[b] Cross-linker: butylene bisacrylamide+styrene.

[c] Porogen: cyclohexanol/dodecanol (91/9), 20 wt% NaCl and 8 wt% starch in water.

[d] Porogen: toluene. Suspension stabiliser: 1 wt% poly(*N*-vinylpyrrolidone) and 2 wt% poly(vinylalcohol) solution in water.

[e] Porogen: cyclohexanol/dodecanol (91/9), 0.1 wt% poly(*N*-vinylpyrrolidone) and 0.2 wt % poly(vinylalcohol) solution in water.

[f] Calculated from pseudo-first-order kinetics.

[g] Calculated from Michaelis-Menten kinetics.

[h] Metal: Zn^{2+}.

[i] Metal: Cu^{2+}.

Using a hybrid covalent/non-covalent imprinting approach, Sellergren and Shea developed polymers incorporating most of the catalytically important features found in chymotrypsin (Fig. 19) [75, 76]. The MIP catalysts were constructed by copolymerisation of MAA, EGDMA and a template monomer consisting of a phenol-imidazole monomer linked via a labile phosphonate ester linkage to a phosphonic acid analogue of BOC-D-phenylalanine. After template removal this would leave behind a site equipped with a mimic of the Ser-His-Asp catalytic triad, a TSA complementary site and a stereoselective binding pocket complementary towards BOC-D-phenylalanine.

The maximum rate enhancement for the hydrolysis of D-*p*-nitrophenyl ester was tenfold greater than the reaction in solution. As expected, the control polymers showed less activity, approximately 5.7-fold or less over the reaction in solution and a complete absence of enantioselectivity. The polymer catalyst showed a 1.85-fold rate enhancement for the D-isomer over the L-isomer; the control polymers (one using an achiral template and without the tetrahedral phosphonate and the other without the phenol-imidazole functionality) showed no preference for one isomer over the other. Notably, modest stereoselective rate enhancements for the hydrolysis of the non-activated ethyl ester were also observed for BOC-D-PheOEt: K_m=1.92 mM, k_{cat}=2.32×10^{-5} min^{-1} and for BOC-L-PheOEt: K_m=1.96 mM, k_{cat}=1.91×10^{-5} min^{-1}. As seen in Table 1, these K_m-values are lower than those observed for the corresponding *p*-nitro-

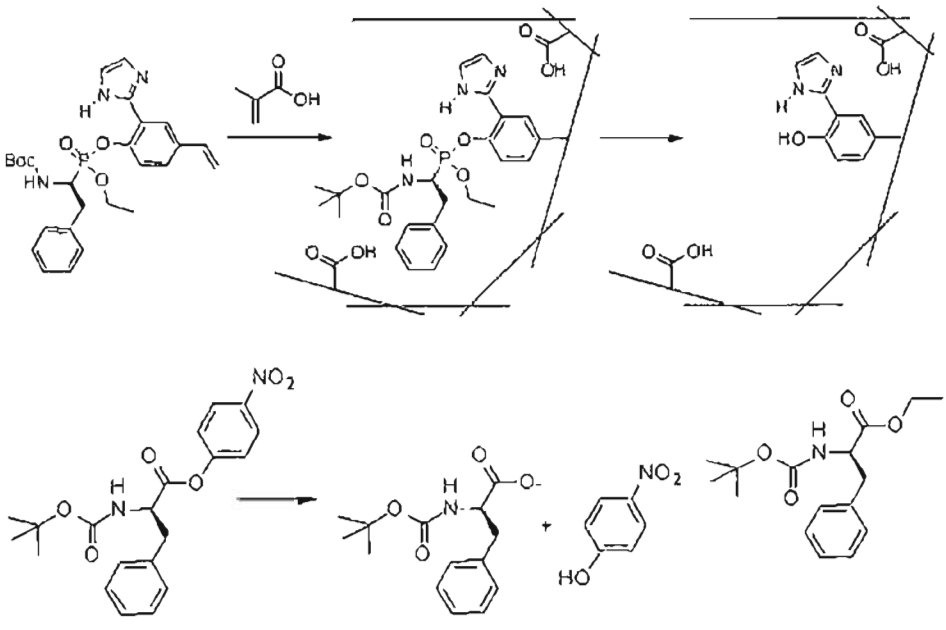


Fig. 19 MIP chymotrypsin mimic prepared by Sellergren and Shea. As substrates either *p*-nitro phenyl ester (*lower left*) or ethyl ester (*lower right*) were used

phenylester substrate (K_m=5.4 mM). This indicates that the ethyl substituent of the TSA template has given rise to a shape complementary site where the ethyl ester fits better than the *p*-nitrophenylester.

Much stronger esterase activity was reported by Wulff and co-workers using a polymerisable amidine, in particular *N,N'*-diethyl(4-vinylphenyl)amidine (7) (see also Sect. 3.1), as functional monomer in combination with a phosphonate TSA template (Fig. 20) [35]. This follows in part the findings that the charged guanidine group of arginine plays an essential role in the mechanism of esterolytic antibodies [77]. The authors reasoned that a complementary shape to the transition state analogue itself may not be sufficient for catalysis and that an appropriately positioned amidine functionality, with similar properties to that of arginine, could be envisaged to provide additional electrostatic stabilisation of the transition state oxyanion. Thus the 7-phosphonate TSA complex provided an "oxyanion hole" for transition state stabilisation similar to that found in serine proteases. This monomer was henceforth used in several systems to create active polymers as described in the following section.

In the first application, the basic hydrolysis of a phenyl ester (Fig. 20) was shown to be accelerated >100-fold in the presence of the MIP catalyst [35]. This was accompanied by Michaelis-Menten kinetics (K_m=0.60 mM; k_{cat}= 0.8×10^{-4} min^{-1}). The low k_{cat} value reflects a poor turnover; in fact, in addition to the template, the product was also found to competitively inhibit the reaction. The postulated mechanism is shown in Fig. 21. The bound substrate (b) is converted into the tetrahedral intermediate (c), which in turn breaks down into the acid and alcohol (d). Product inhibition was attributed to the carboxylate group (X=CH_2), which binds strongly to the amidine residue (see Sect. 3.1).

To avoid the product inhibition in further studies Wulff et al. investigated the hydrolysis of carbonates and carbamates, which liberate CO_2 and alcohols, with no significant affinity for the amidine site [37]. Thus, the hydrolysis of diphenyl carbonate and diphenyl carbamate were catalysed by the MIP (imprinted with diphenyl phosphate and DEVPA) with rate enhancements of 588 and 1435,

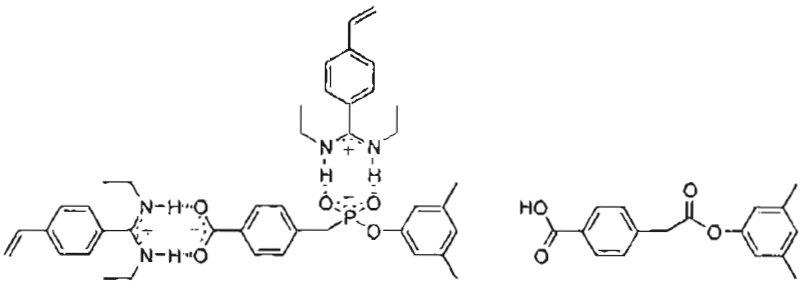


Fig. 20 *Left*: Two equivalents of 7 forming hydrogen-bonded ion pairs with one equivalent of TSA. *Right*: Phenylester substrate

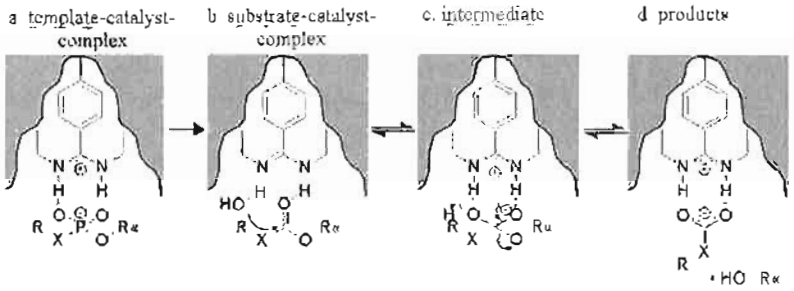


Fig. 21 Proposed mechanism of the basic hydrolysis of esters, carbonates and carbamates (X=CH_2, O, NH) in the cavity

respectively, compared to the rates of the uncatalysed reactions. Relative to that of the control polymer, the MIP catalyst showed rate enhancements of 10 and 24, respectively.

As an alternative to the classical technique to produce MIPs as crushed monoliths, an aqueous suspension polymerisation technique for creating MIPs in bead form was introduced (see also Sect. 3.1) [78]. The pseudo-first order rate constants showed enhancements by factors of 293 for carbonate and 160 for carbamate, compared to the uncatalysed reactions. These results were somewhat poorer than those given by the MIPs produced as crushed monoliths. On the other hand, the rate enhancements with respect to the control polymer were 24-fold for carbonate and 11-fold for the carbamate, which showed an improvement compared to the enhancements obtained with the crushed monoliths (tenfold for carbonate; 5.8-fold for carbamate).

Again based on 7, Emgenbroich and Wulff recently reported on an enzyme model exhibiting enantioselective esterase activity (Fig. 22) [79]. Two enantiomerically pure stable α-amino phosphonic monoesters (L-LeuP and L-ValP) were connected by stoichiometric non-covalent interactions to two equivalents of 7. The complex was thereafter copolymerised with EDMA and the resulting polymer freed from template generating enantioselective catalytic sites. The

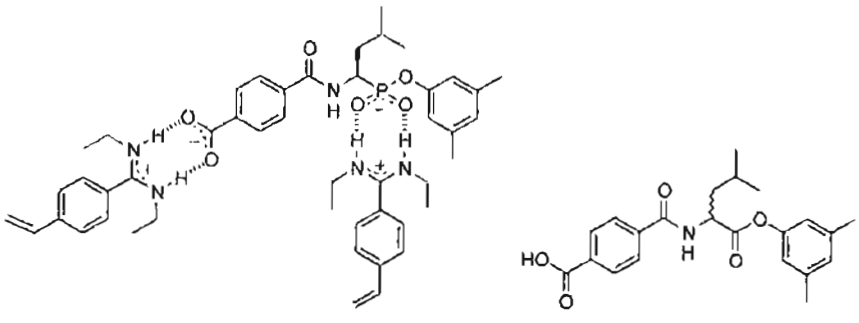


Fig. 22 Complex of chiral TSA L-LeuP with 7 and chiral substrate

catalyst prepared by imprinting of L-LeuP enhanced the hydrolysis of the corresponding substrate L-Leu by a factor of 325 relative to that of a buffered solution. Relative to a control polymer, the enhancement was still about 80-fold, showing one of highest imprinting effects in MIP-based catalysis. The polymers exhibited Michaelis-Menten kinetics, allowing the Michaelis constant K_M and the catalytic constant k_{cat} to be estimated. The ratio of the catalytic efficiency k_{cat}/K_M between the hydrolysis of the two enantiomers, representing the enantioselectivity, was 1.65. This derives from both selective binding of the substrate (K_ML/K_MD=0.82), and selective formation of the transition state (k_{cat}L/k_{cat}D=1.36). Thus, these catalysts show good catalysis together with high imprinting and substrate selectivity. They also showed strong competitive inhibition caused by the template, which further reflects the enzyme-analogue behaviour of the model.

Based on a similar principle, Resmini et al. developed guanidine functionalised soluble polymer microgels imprinted with a TSA for the hydrolysis of activated carbonates [82]. In contrast to the heterogeneous systems the resulting gel was soluble in DMSO/buffer 9:1 allowing direct monitoring of the kinetics by UV-Vis spectroscopy. The Michaelis-Menten kinetics, for the hydrolysis of the *p*-nitro phenyl carbonate, gave a rate enhancement of k_{cat}/k_{uncat}=530.

The inapplicability of water-soluble substrates and the mass transfer problems with conventional MIPs prompted Goto et al. to investigate a "surface molecular imprinting technique" [83]. Briefly, the MIP was prepared by polymerising water-in-oil (W/O) emulsions containing the functional host molecule (oleyl imidazole), the template (N-*t*-Boc-L-histidine) and the cross-linking monomer (DVB). Co^{2+} ions were used to coordinate the imidazole residues of the host molecule. The host-guest complex was formed at the interior surface of the water droplets, and the surrounding organic layer was polymerised. Subsequently the ability of the monoliths to catalyse the hydrolysis of N-*t*-Boc-L-alanine *p*-nitrophenyl ester was investigated. Using a substrate analogue a 1.8-fold rate enhancement was found for the imprinted polymer over the control.

The latest generation of catalytic MIPs from Wulff's group mimic carboxypeptidase A, an exopeptidase catalysing the hydrolysis of C-terminal peptides [58, 59]. The enzyme contains a stereoselective binding site complementary to peptides containing C-terminal large hydrophobic amino acids and nearby a catalytically important zinc ion coordinating the water or hydroxide for the hydrolysis reaction. The bio-insipred catalytically-active monomer is again based on the amidine monomer 7 but with one of the *N*-ethyl groups substituted with a chelating ligand site for Zn^{2+} or Cu^{2+} (Fig. 23). By using a pyridyl-containing TSA that can form a coordinative bond to the metal a catalytically-active polymer was prepared by polymerising the complex with EDMA as cross-linking monomer.

The resulting activities are very high and show rate enhancements of up to 3,264 compared to the background reaction in solution. However, the imprinting efficiency, compared to the control polymer, is only 62. According to the mechanism, the authors assumed that the Zn^{2+} ion coordinates to a water mole-

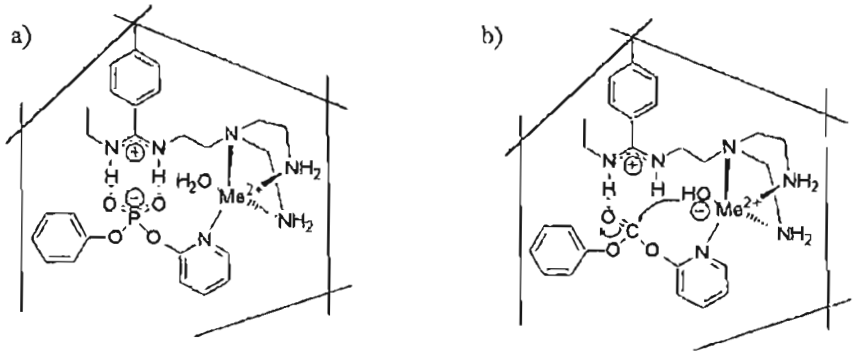


Fig. 23 Preparation and function of imprinted carboxypeptidase A mimic by Wulff et al. a Cavity imprinted with the template and functional monomer in presence of Me^{2+} (Me=Zn, Cu). b Substrate carbonate in the cavity attacked by the metal-coordinated hydroxide

cule, causing an increase of the acidity of the water. The resultant α-coordinated hydroxy groups can then attack the substrate. In a following paper they changed the metal ion to Cu^{2+} [59]. This change led to further dramatic effects in the catalytic enhancement of the resulting polymer, up to a $k_{MIP}/k_{solution} \approx 77{,}000$. By comparing with a control polymer they detected an imprinting effect of 80, whereby only a small part of the catalysis is caused by the imprinting effect. Also, the enzyme-like catalysis showed extraordinary turnover numbers with $k_{cat}=28\ min^{-1}$, thus outperforming all previous MIP-based catalysts. By comparing the Michaelis-Menten kinetics for the polymer with the background reaction a catalytic activity k_{cat}/k_{uncat} of up to 110,000 was calculated. For carbonate hydrolysis this is the fastest enzyme mimic presented and is even one order of magnitude better than catalytic antibodies, although, admittedly, the antibodies exhibit higher rate enhancements compared to controls.

4.2 Chemoinspired catalytically active MIPs – Imprints of metal catalysts or their analogues

An alternative approach to imprinted catalysts is via the incorporation of a low molecular weight catalyst that already exhibits activity in solution. To achieve this the catalyst needs polymerisable groups that are located remote from the active centre. Next, a complex is formed with a suitable template molecule. The template may be the substrate (or analogue), a TSA or the product (or analogue) of the reaction. This depends on the reaction, the accessibility of the required molecules and their ability to coordinate to the catalyst. By imprinting such a complex, a cavity is formed that is complementary to the catalyst and the bound template. After template removal, the formed cavity supports the binding and recognition of the substrate molecules. Thus, in addition to simple catalyst immobilisation, the formed cavity introduces shape selectivity,

which may also result in enhanced activities. Clearly, given the wide field of metallo-enzymes, this approach may also be classified as "bio-inspired" (see previous section). However, the literature reports so far are based on metall-organic complexes, which exhibit high solution activity per se.

The first examples of the combination of inorganic catalysts with the imprinting technique were published in the mid 1990s. The group of Lemaire presented a polyurethane-supported Rh-catalyst, imprinted to promote hydride transfer to form alcohols by using the product as template (1-(*S*)-phenylethanolate) [84, 85]. The enatioselectivity of the homogeneous catalyst of maximum 67% (e.e.) was hereby slightly improved (e.e. 70%) by the cross-linked polymer support. Without cross-linking the catalyst was less effective.

Severin et al. reported on a defined polymerisable ruthenium-complex with a TSA template as one of the ligands (Fig. 24) [86, 87]. The crystal structure indicated the phosphinic acid complex to be analogous to the six-membered transition state of the transfer hydrogenation catalysed by Ru. After incorporation of the complex into a polymer matrix, then template removal, the resulting imprinted catalyst was active in catalysing the hydrogenation of aromatic ketones. The selectivity of the MIP was demonstrated with a competition experiment with seven similar substrates where the substrate, being the analogue of the TSA, showed the highest activity.

In a further development of these catalysts the authors used a rhodium(III)-complex with a chiral chelating ligand [88]. The resulting MIPs (Fig. 25) showed a high enantioselctivity (up to 95% e.e.) for the transfer hydrogenation of acetophenone derivatives, whereas the control polymer imprinted without the TSA showed similar e.e. but only half the yield compared to the MIP.

In an effort to enhance the stereoselectivity of the platinum catalysed ene reaction shown in Fig. 26, Gagné and co-workers synthesised platinium(II) complexes between a polymerisable chiral diphosphine ligand and chiral binaphtol (BINOL) ligands [89]. Removal of BINOL would leave behind a chiral BINOL shaped cavity. This resulted in an immobilised precatalyst that could be

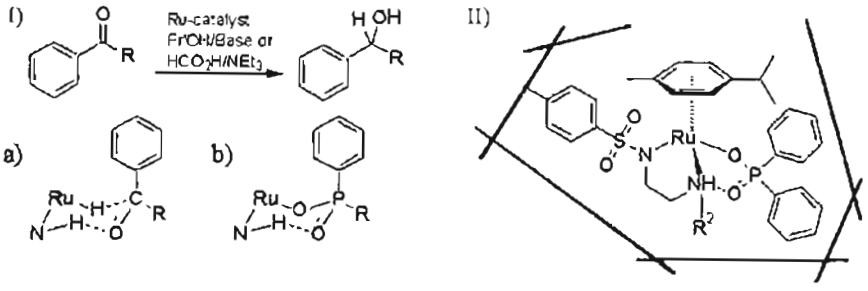


Fig. 24 I) Hydrogen transfer to aromatic ketones catalysed by ruthenium half-sandwich complexes. a) Proposed transition structure. b) TSA mimicked by the phosphinato complex. II) Imprinted Ru-catalyst in complex with TSA-template (R^2=H, $CH_2C_6H_4CH{=}CH_2$)

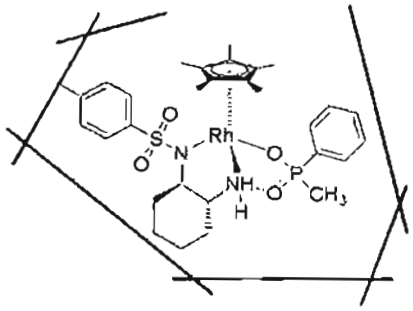


Fig. 25 TSA mimicked by the phosphinato complex imprinted Rh-catalyst in complex with TSA template

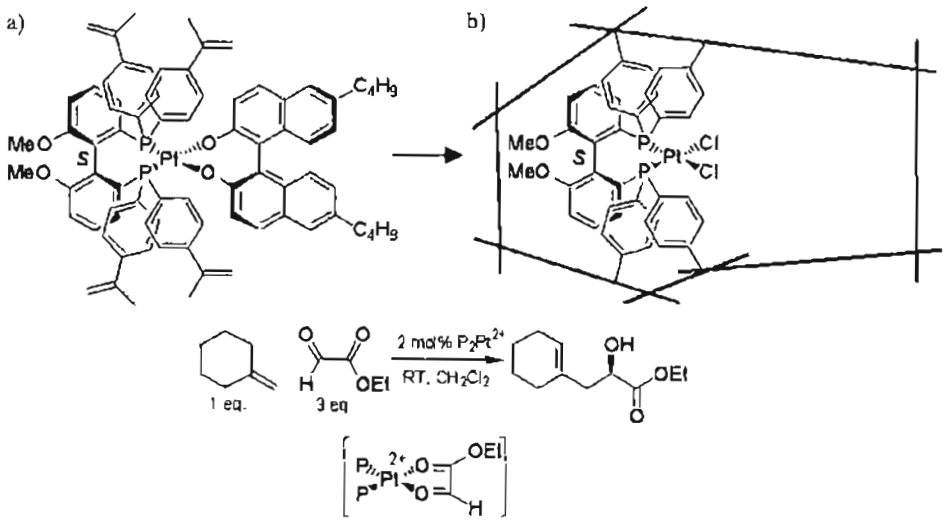


Fig. 26 a) Polymerisable catalyst complex with *S*-BINOL as template ligand. b) Empty cavity remaining after template removal. After activation the polymer can catalyse the shown ene reaction

activated for asymmetric catalysis. Poisoning experiments using the chiral poison (*R*)- or (*S*)-1,1′-binaphthyl-2,2′-diamine showed that the generated active sites exhibited similar reactivity and selectivity for the ene reaction. However, the enantioselectivity was unfortunately poor, reflecting the relatively large influence of the chiral diphosphine ligand in controlling the enantioselectivity of the reaction rather than the cavity shape.

Cammidge and Gagne have recently reported molecularly imprinted catalysts for the Suzuki coupling shown in Fig. 27. In both approaches palladium catalysts containing phosphine ligands were imprinted. Cammidge et al. used

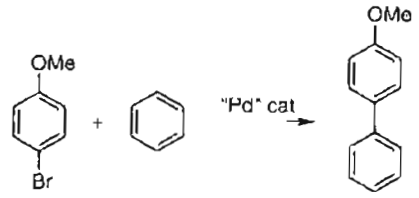


Fig. 27 Suzuki reaction catalysed by Pd-MIPs

a Pd-complex with two polymerisable phosphine ligands and a benzene-1,2-diol as template ligand [90]. The imprinted catalyst gave a higher yield (81%) compared to the homogeneous catalyst (56%). Importantly, the catalyst was reusable with no reduction in the yield, whereas the homogeneous catalyst lost activity on repeated usage (45% at second use).

Gagne et al. used a bipodal phosphine ligand and 4,6-dinitrobenzene-1,2,3-triol as a template ligand [91]. In addition to this complex a primary amine, stabilised by a polymerisable crown ether, was added to form a polymerisable ternary complex via non-covalent interactions, thus creating a hybrid crown-ether functionalised active site during the imprinting step (Fig. 28). Using this original approach they improved the activity of the resulting MIP catalyst by factors of up to 2.5.

5 Perspectives

The number of reports of the use of "non-traditional" functional monomers in molecular imprinting has grown steadily in recent years. While we hope to

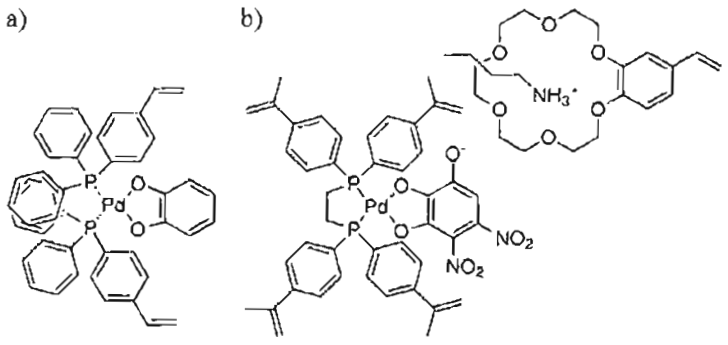


Fig. 28 Pd-catalysts complexed with template for the catalysis of Suzuki couplings by a) two phosphine ligands with benzene-1,2-diol as template and b) a chelating bisphosphine ligand with 4,6-dinitro-benzene-1,2,3-triol as template in a ternary complex with a primary amine stabilised by a crown ether

have demonstrated that there are obvious benefits to be gained from the preparation of new binding monomers for both recognition and catalysis, such developments are still at an early stage within the field. Undoubtedly this will change in the near future as more researchers in the field discover the advantages in binding site homogeneity and affinity that these monomers can bring to their macromolecular receptors. The recent examples of MIPs exhibiting enzyme-like catalysis convincingly demonstrate these benefits. By combining the type of chemistry described in this review for construction of the binding or catalytic sites with techniques to generate the polymer as beads, nanoparticles, microgels or thin films we envisage a new generation of imprinted receptors or catalysts exhibiting strong improvements compared to state-of-the-art materials.

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Molecular Imprinting: State of the Art and Perspectives

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Abstract Molecular recognition is central to how biological systems work. The molecular imprinting technique is a valuable polymerisation method for preparing synthetic materials able to mimic the molecular recognition phenomena present in living systems. A molecule that acts as a template is associated with functional monomers to form a complex by means of covalent linkages or noncovalent interactions. A polymerisation-crosslinking reaction is then performed around this complex. Upon removal of the template species, functionalised cavities, that have memorized the special features and bonding preferences of the template, are left inside the polymer network. A large number of potential applications for this class materials are being intensively developed, for example as chromatographic stationary phases

or as stereospecific catalyst. To improve this technique, the challenge is now to rationalize the necessary stiffness of the network with the expansion of its capacity. From this perspective, the use of materials involving supramolecular organisation is of great interest to bring closer to the biological processes and so to improve the recognition properties.

Keywords Molecular recognition · Imprinting · Polymer · Chromatography · Catalysis

1 Introduction

Molecular recognition is central to how biological systems work, especially at the cellular level (example Fig. 1). The observation of the various systems where processes of recognition occur (enzyme substrate complexes, antibody-antigen systems, DNA replication, membrane receptors, and so on) has indicated a certain number of directions for the preparation of synthetic systems capable of molecular recognition.

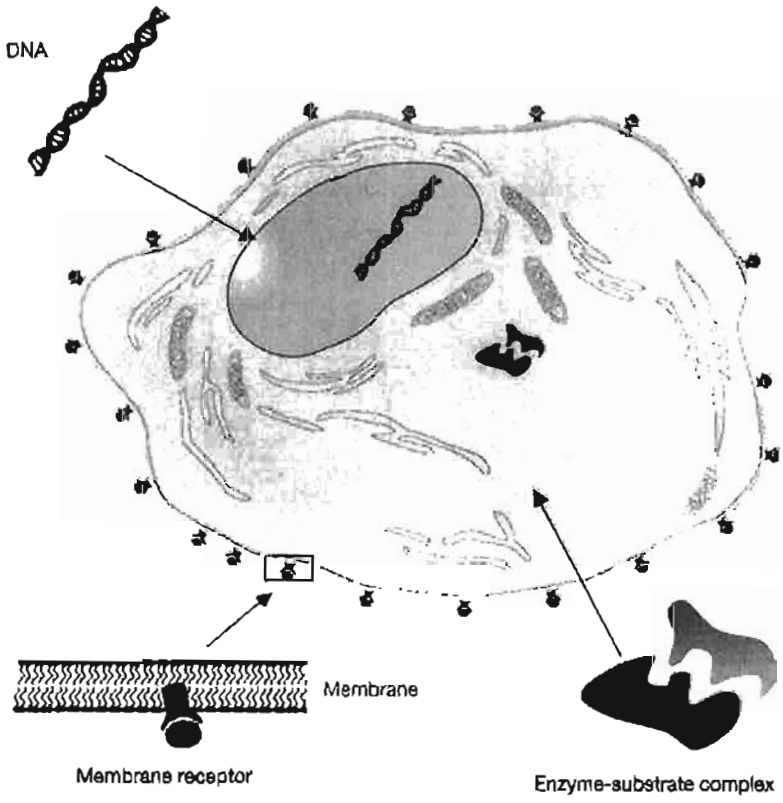


Fig. 1 Examples of molecular recognition systems occurring in the cell

Fig. 2 Changes that occur in the shape of the enzyme when the substrate binds to the active site so that catalysis can take place

Let us consider for instance, the way enzyme-substrate type systems operate. In 1894, Emil Fisher developed the "lock-and-key" notion [1], which has become one of the most frequently mentioned concepts in Life Sciences. This model describes how proteins bear specific cavities into which a given substrate fits. The process was refined by Koshland [2] who considered that during the interaction phase, the geometry of the cavities became adapted to the substrate, optimising the interactions taking place (for example Fig. 2). In spite of its apparent complexity, the recognition process involves simple interactions between chemical groups – the overall exact location of the interactions leading to molecular recognition.

Considerable efforts have been made to synthesise systems mimicking the natural processes of molecular recognition [3, 4]. The first studies involved the preparation of small ring systems and 'cage' molecules that could become specifically associated to a given ion or to small molecules: crown ethers [5], cryptates [6, 7], cyclophanes [8], and cyclodextrins [9] (Fig. 3). Abzymes have been designed in an attempt to study recognition of more complex molecules [10]. However, they were found to be difficult to prepare and to use, and their cost is generally high.

Antibodies, receptors and enzymes are biomolecules frequently used in analytical chemistry and biochemistry in various applications aiming to detect

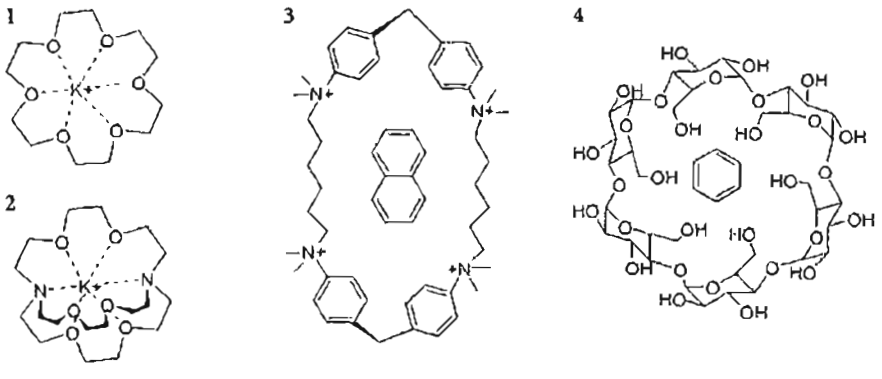


Fig. 3 Examples of synthetic receptors capable of molecular recognition: (1) crown ether; (2) cryptate; (3) cyclophane; (4) α-cyclodextrin (adapted from [5–9])

and quantify a host molecule (biosensors, affinity chromatography supports, immunoanalysis kits, etc.). These applications share a common first step where the guest molecule is recognised and selectively adsorbed by the receptor biomolecule. However, biomolecules can only be used in experimental conditions close to those found in nature, limiting their fields of application. Their replacement by more stable, cheaper materials with similar selectivity therefore became attractive.

The techniques for the synthesis of molecularly imprinted polymer (MIP) then developed. The principle consists of polymerising and crosslinking functional monomers, previously positioned by low-energy or covalent interactions around a molecule – the guest or template molecule – so as to "freeze" the imprint. Following extraction of the template molecule, the remaining three-dimensional network presents pores with a geometry and positioning of the functional groups complementary to those of the template. This enables it to specifically recognise the template molecule.

The conception of this technique was originally inspired by the works of Linus Pauling in the 1940s outlining the possible mode of action of antibodies [11]. One of his students, F.H. Dickey, was the first to prepare selective adsorbents by precipitating silica gel in the presence of dyes (methyl orange and its derivatives) [12]. When washed, the materials presented an enhanced affinity for the dyes. Similarly, materials used for chiral recognition [13] or for pesticide separation were studied. However, owing to the low separating powers obtained and the short useful life-time of the materials, this line of research was to be finally abandoned [14]. It was only with the works of G. Wulff from 1972 [15], followed by those of Mosbach in 1984 [16], that the principles of the technique of molecular imprinting were laid down.

The simplicity of use, the relatively low cost and the broad range of possible guest molecules (small organic molecules, ions but also biological macromolecules) have since led to the important development of this technique, as illustrated by the increasing numbers of publications over recent years [17–25]. The fields of application of these imprinted polymer networks are very diverse. We can mention chromatographic supports (particularly for the separation of enantiomers) recognition elements in the preparation of specific sensors, catalysts, systems for stereospecific synthesis, and selective adsorbents.

2
MIP Materials

The principle of molecularly imprinted polymer (MIP) production is schematised in Fig. 4.

The preparation of the materials starts by positioning the functional monomers around a template molecule. The monomers interact with sites on the template via interactions that can be reversible covalent or non-covalent (hydrogen, ionic, Van der Waals, π-π, etc.). They are then polymerised and cross-

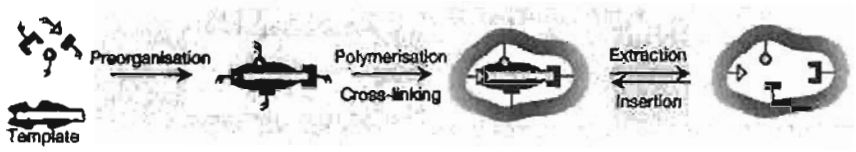


Fig. 4 Principle of the molecular imprinting technique

linked around the template in order to fix their position and to "freeze" the geometry of the pores in the network. The template molecule is then extracted, leaving a polymer with functional sites capable of molecular recognition.

2.1 The Different Stages Involved in the Preparation of the Materials

2.1.1 Preorganisation Stage

The development of an imprinted network starts with the choice of the template molecule. It is this molecule that the material will keep in memory. The choice remains limited, however, by the functional monomers available and susceptible to interact. Depending on the domain studied, diverse molecules have been used (Fig. 5): amino acids [26], sugar derivatives [27], steroids [28], nucleotides [29], pesticides [30], dyes [12], drugs [31–36], metal ions [37–39], more inert molecules (like anthracene, benzene and its derivatives) [40–42], and even more complex molecules such as proteins or enzymes [43, 44].

Note that with chiral guest molecules for which it is difficult to obtain a pure enantiomer, the use of a chiral functional monomer, interacting preferentially with one of the two enantiomers in a racemic mixture, can be an interesting approach [45].

Several types of monomers have been used for this (Fig. 6), especially easily polymerisable molecules. Acrylate monomers [46–51] and acrylamides have been used the most [52–56]. Systems with polystyrene [47, 57–63] and polysiloxane [64] backbones have also been synthesised. Recently, other types of polymer such as polyurethanes [65], polypyrroles [66–68], and polyimidazoles [69, 70] have been used in special applications. Renewed interest has also been shown in silicas and sol-gel glasses [71].

In the first step, the contact occurring between the functionalised monomers and the template molecule leads to the formation of a complex. Its structure and stability will then determine the behaviour of the future MIP. The interactions involved must be sufficiently strong to remain intact during the polymerisation stage but sufficiently labile to enable template extraction and reinsertion of guest molecules in the later stages. These interactions must therefore occur rapidly and be reversible. It is crucial to optimise the choice of the different components of the system at this point.

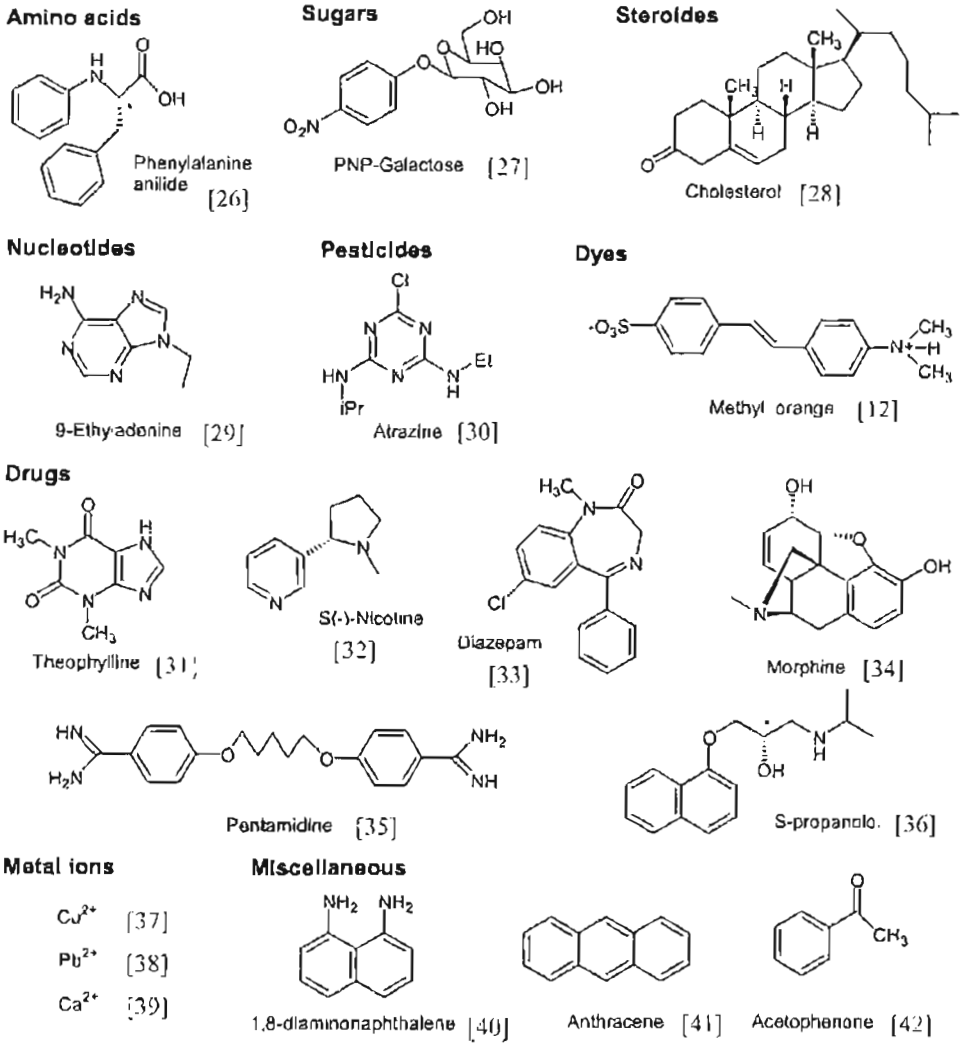


Fig. 5 Some examples of molecules used as templates in MIP

Different types of strategies can be distinguished depending on whether the bonding between template and host is covalent or not:

1. Covalent interactions (Fig. 7): this approach involves the formation of an easily cleavable functionalised monomer-template complex [61, 18]. The first example of an imprinted polymer network was prepared by Wulff's group and used the reversible formation of an ester bond between a diol and 4-vinylphenylboronic acid [15]. The reaction rates to reach equilibrium between the ester and boronic acid are comparable to those obtained with

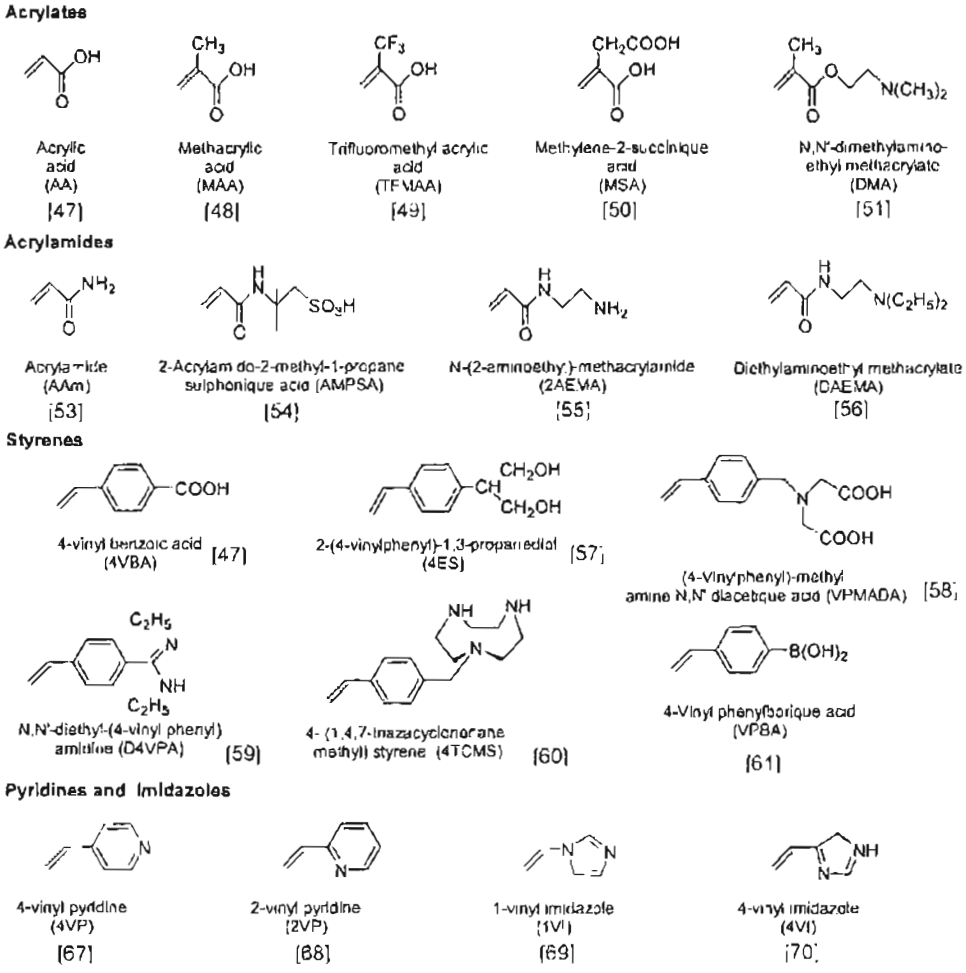


Fig. 6 The monomers most commonly used in MIP

non-covalent systems [72]. It can be noted that systems using two different boronic acids to interact with the template lead to MIPs [73] that ensure higher resolution of racemic mixtures than those consisting of a single boronic acid [74].

Covalent bonds involving Schiff bases [75], esters [76], amides [77], and ketals [57, 78] have also been used. However, their much slower monomer-template interaction kinetics have excluded them from several types of application.

Although this covalent bonding strategy provides well-defined cavities, the limited choice of functional monomers and hence of useable template molecules has restricted its use.

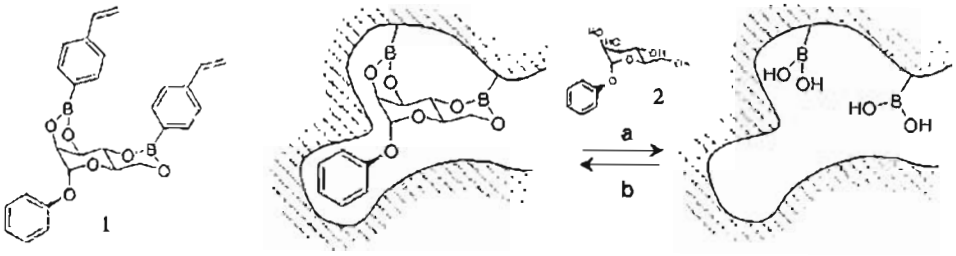


Fig. 7 Schematic representation of a cavity obtained by polymerisation of 1. The interactions occurring between the network and the guest molecule 2 are covalent. The guest is extracted in the presence of water and methanol (a), and its reinsertion leads to the reoccupation of the imprinted cavity (b) (adapted from [61])

2. Non-covalent interactions: this approach is simpler to use and can be adapted to a larger range of template molecules. Here, the guest interacts with one or several functional monomers [79–82] to form a complex. The stability of the complex depends on the different components present in the mixture. The forces involved can include ionic interactions [83], hydrogen bonding [54], π-π interactions [84] and hydrophobic interactions [85]. Owing to the lower stability of the complex that is formed, systems based on these types of interactions often lead to MIP that are less specific than those that use covalent interactions. On the other hand, the host-guest exchange rates are more rapid and the MIP are suitable for use in chromatography.
Among the most frequently used monomers we can mention carboxylic acids (e.g. acrylic, methacrylic and vinyl benzoic acids), sulfonic acids and heterocyclic bases (e.g. vinylpyridines, vinylimidazoles).
Currently, great efforts are being made to broaden the range of templates that can be used and to improve the specificity of 'non-covalent' MIP. The first systems developed were not very selective [79]. It was only once the experimental conditions had been optimised (conditions of synthesis, host/guest ratios, eluent, etc.) that Mosbach et al. achieved materials with selectivities similar to those obtained with the covalent approach [48]. Owing to the weakness of the interactions occurring, a large excess of functional monomer must be added to shift the equilibrium towards formation of the complex (typical monomer:guest ratios are 4:1). This leads to the formation, inside the imprinted network, of sites with different affinities for the molecule to be recognised (polyclonality) and lowers the specificity of the MIP. In chromatographic applications, for instance, this causes broadening of the peaks and decreased resolution. To overcome this problem, new monomers interacting more specifically with the template at several points have been developed for addition in stoichiometric quantities [59, 86] (Fig. 8).
Template molecules with moieties capable of binding to a metal can also be incorporated in a MIP, if the metal presents polymerisable ligands. The strength of the metal-template bond can be easily modulated by adjusting

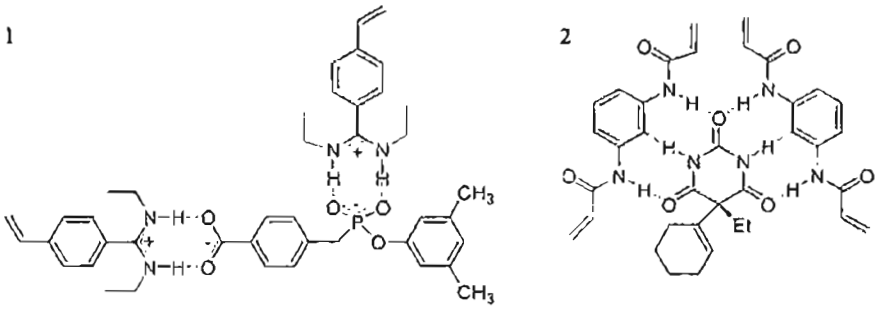


Fig. 8 Functionalised monomer/template molecule complexes based on strong non-covalent interactions (the various molecules are added in stoichiometric proportions): (1) adapted from [59]; (2) adapted from [86]

the experimental conditions. This approach was recently evaluated and gives very good results, in some cases equivalent to those obtained with enzyme systems [58, 60, 87].

3. Finally, there is an alternative strategy which uses a double approach: covalent plus non-covalent (Fig. 9). With the example of cholesterol taken as template molecule, M.J. Whitcombe and co-workers esterified the hydroxyl function, to enable incorporation of the cholesterol within the polymer network. After breaking the ester bond, the template only acted through non-covalent interactions [28]. This strategy, which was also used by A. Sahran [88, 89] does present the disadvantage of potentially modifying the geometry of the imprinted site following hydrolysis.

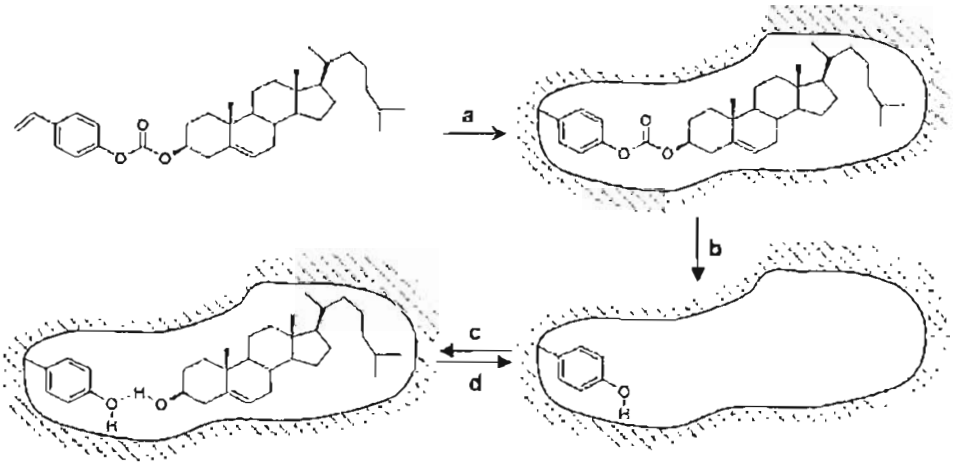


Fig. 9 Preparation of a MIP using covalent interactions during the polymerisation-cross-linking step (a) and the extraction of the template molecule (b). During the specificity tests, (c) and (d), the interactions occurring are of the hydrogen bonding type (adapted from [28])

2.1.2
Step of Polymerisation-Crosslinking of the Monomers Around the Complex Formed in the First Step

Polymerisation mode [90]. Owing to its ease of use, radical polymerisation is the most frequent. The crucial question is to determine how to carry out this polymerisation-crosslinking step with minimum disturbance of the complex already in place. Choices must be made, for instance in radical polymerisation, the radicals can be generated at 60 °C (α,α′-azoisobutyronitrile – AIBN) or 45 °C (with azobis valeronitrile – ABDV) which could cause heat destabilisation of the complex, or at 4 °C with low-temperature photochemical radical production (AIBN, 360 nm). Comparative studies on recognition specificity have shown that the photochemical approach gives the most specific materials [91].

Crosslinking. To date, only a small number of crosslinkers have been tested as their poor miscibility with the monomers considerably limits the choice (Fig. 10) [92–99]. The most frequently used crosslinkers in acrylate systems are acrylate esters of diols and triols such as ethylene glycol dimethacrylate and trimethylolpropane trimethacrylate [92–94]. In styrene-based systems, isomers of divinylbenzene are usually used [61a, 62].

Wulff et al. studied the influence of the degree of crosslinking on the suitability of a MIP used as a chromatographic support to separate two enantiomers – one of which had been used as the template [94]. The results obtained are reported in Fig. 11, selectivity being represented by values of separation factor α greater than 1. Irrespective of the crosslinker used, to obtain significant recognition of the guest molecule, the network has to be strongly crosslinked to limit chain relaxation [94]. For similar proportions of crosslinker, the use of a short molecule (ethylene glycol dimethacrylate rather than tetramethylene

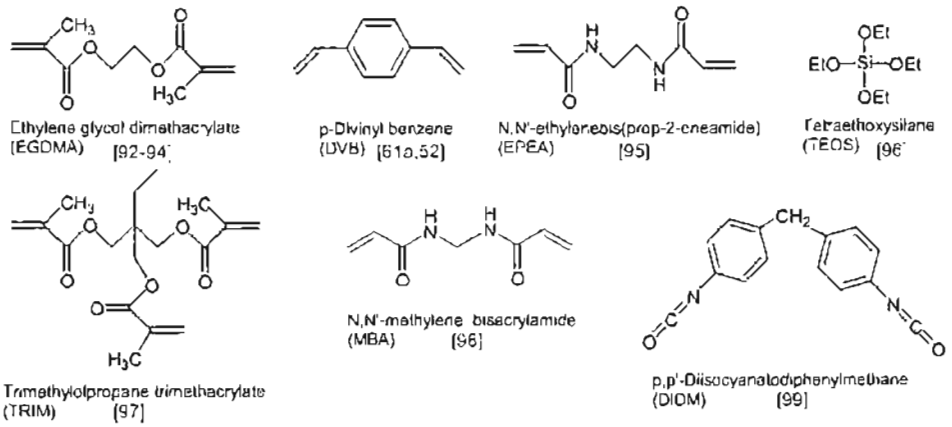


Fig. 10 Some of the crosslinkers used to prepare MIP

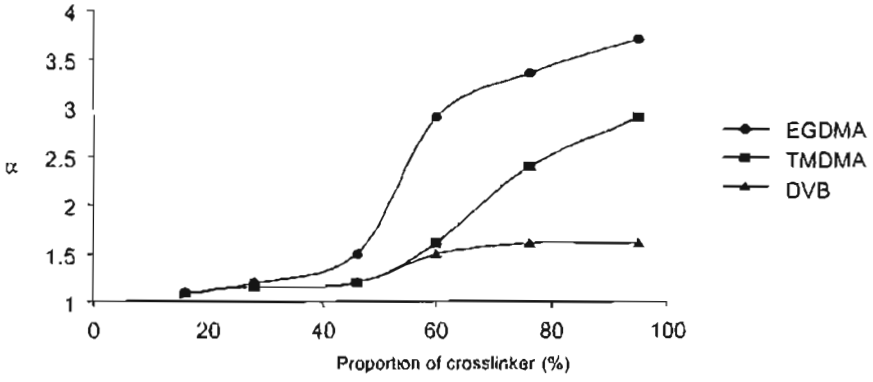


Fig. 11 Influence of the proportion of crosslinker on the recognition specificity of MIPs. The crosslinkers represented are ethylene glycol dimethacrylate (EGDMA), tetramethylene dimethacrylate (TMDMA) and divinyl benzene (DVB) (adapted from [94])

dimethacrylate) but not too rigid (ethylene glycol dimethacrylate rather than divinyl benzene) leads to more specific recognition. So, a high degree of crosslinking appears necessary and in most cases over 70 mol.% crosslinker is required.

While a high degree of crosslinking enhances the mechanical stability of the MIP, resulting in reduced deformation of the imprinted sites, it does rigidify the network, deteriorating the extraction and insertion kinetics of the guest molecule and reducing the accessibility of the imprinted sites in the network. So, in networks based on non-covalent interactions, at best 10% of the sites formed are active. The result is that the high proportions of crosslinker bring about a decrease in the capacity of the network meaning that the MIPs can only be basically used for analytical purposes. In an attempt to alleviate this shortcoming, it has been suggested that tri- and even tetravalent crosslinkers be used [100, 101]. Experiments have also been carried out with crosslinkers able to interact in the same way as functionalised monomers [86].

Shaping the MIPs. The use intended for the MIP will determine its shape. The most widely used technique consists of preparing a bulk polymer which is then ground and graded. The particles obtained (Fig. 12(1)) can then be used in various applications [68, 102, 103].

To use MIPs in chromatography, other techniques have been developed. Polymers have been prepared in situ in chromatographic columns [104] and in capillary systems for electrophoresis [105]. Their performance does not, however, come up to expectations and efforts have been made to obtain more homogeneous materials. This was done in two ways: i) grafting or covering preformed particles by the MIP. The particles covered can be silica or trimethylolpropane trimethacrylate [52, 106–108]. Sveç and Fréchet used a process they call two-step swelling. The first step consists of making polystyrene support beads and the

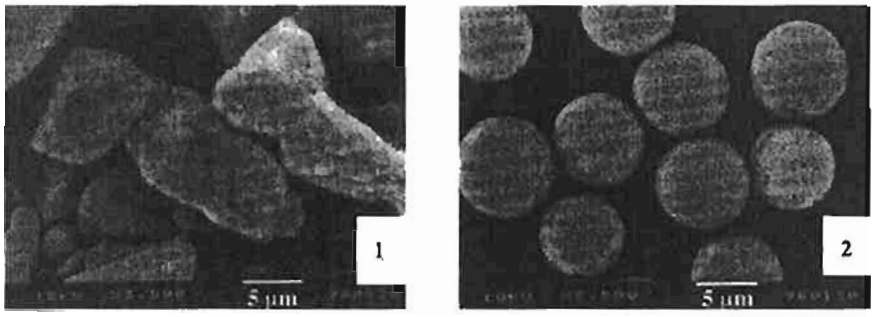


Fig. 12 Scanning Electron Microscopy images of MIP used as stationary phase in chromatography: (1) MIP particles obtained by grinding, grading and sedimentation; (2) beads prepared by an emulsion process (from [114])

second is the polymerisation around them of the MIP, leading to the formation of perfectly spherical beads [109] and ii) the preparation of polymer beads via processes in emulsion or in suspension [110–114]. Spherical imprinted particles with a very narrow size distribution are then obtained, and can be used as high performance chromatography stationary phase (Fig. 12(2)).

The formation of thin layers and membranes, for analytical applications or as sensors, has also been studied. Here, either the MIP is grown directly on a surface (for instance an electrode) [115], or particles of MIP are held together by a binder to generate a material similar to those used for thin layer chromatography [116]. The first method avoids the grinding step and thus reduces the risk of deforming the sites.

A surface imprinting technique has also been investigated [117–122]: after the template molecule has been dissolved in the presence of functionalised monomer, the preformed complex is grafted onto an activated surface (silica or a glass surface). This approach can prove to be particularly interesting for the recognition of macromolecules such as proteins which present much greater problems of insertion and extraction than small molecules [123]. Using similar techniques, metal adsorption sites can be integrated onto surfaces [124].

Finally, a new method for obtaining permeable MIPs has recently been developed: first, the guest molecule is grafted onto a very porous material (support network), then polymerisation is carried out around it. The support network is then destroyed leaving a highly porous MIP suitable for chromatographic applications [125].

2.1.3
Template Molecule Extraction Stage

The proportion of extraction of the template molecules interacting with the MIP via easily hydrolysable covalent bonds or non-covalent linkages is estimated to be about 90% [17]. The remaining molecules are trapped in highly

crosslinked zones. This problem is exacerbated with macromolecules where steric hindrance lowers the efficiency of extraction.

In addition, the synthesis of MIPs requires large quantities of guest molecule (50 to 500 µmoles per gram of polymer). So, when pure template molecule is difficult or expensive to obtain, reaching quantitative template extraction yields can be primordial. Extraction conditions must then be optimised to obtain yields of over 99% [126, 127].

The extraction step uses an appropriate solvent. It often proves to be long and the actual process involved is dependent on the system in question. So, automation of the washing steps for industrial applications still remains problematic.

Extraction of the template leaves a three-dimensional material in which the cavity shapes and functional group locations are complementary to the guest molecule.

2.2 Influence of the Solvent

Although this parameter has been little studied, the choice of solvent is also particularly important in optimising the properties of MIPs:

- Particularly for non-covalent systems, in the steps of pre-organisation and polymerisation, the solvent plays an essential role in that it influences the type and the strength of the interactions occurring. The interactions are strongly dependent on the polarity and the dissociating power of the solvent. In the example of a MIP composed of ethylene glycol dimethacrylate (EGDMA) and methacrylic acid (MAA) polymerised around atrazine, the best performance was obtained with fairly apolar solvents such as toluene and dichloromethane [128]. MIPs based on covalent interactions, on the other hand, are only slightly affected by the type of solvent used [129].
- In addition, the solvent generates pores and, in conjunction with other parameters such as temperature, influences the morphology of the material (size, shape and size distribution of the cavities). For chromatographic applications, macroporous networks are preferable. As the pores are more accessible, recognition is enhanced and retention times reduced. For instance, the use of acetonitrile as solvent in acrylate networks leads to a more macroporous structure than chloroform [130].
- When testing the properties of the MIP, the solvent modifies the morphology of the network through swelling processes. For instance, in acrylate systems, more swelling occurs in chlorinated solvents such as chloroform and dichloromethane than in tetrahydrofuran or acetonitrile. The swelling can decrease the recognition capacity. It is therefore often judicious to use an analytical solvent with a structure as close as possible to that of the synthesis solvent so as to create a micro-environment identical to that predominating when the network was originally prepared [131].

Most of the results obtained to date concern MIPs synthesised in organic media. They should now be transposed to aqueous systems which have a much broader field of application (protein recognition) and perspectives of development [132, 133]. This poses a certain number of problems since the interactions occurring in the two types of system are fundamentally different (Van der Waals interactions for organic systems and hydrophobic interactions and complexation in aqueous media [134]).

3
Recognition Properties

3.1
Guest Capacity

As mentioned above, a certain number of sites in the MIP remain inaccessible. This can be due to the presence of residual template molecules, excessive crosslinking limiting diffusion, or deformation of the network. In covalent systems, 80 to 90% of the sites vacated can be occupied again; in non-covalent systems, the proportion of reinsertion falls to 10 to 15% [135]. Non-covalent systems therefore provide low-capacity materials. This difference of behaviour is apparent from Fig. 13 [136b,c].

After extraction of the template, any deformation of the recognition sites, due to swelling of the MIP by solvent or relaxation of the polymer chains can hinder reinsertion. If the guest molecule is bound by covalent interactions (Fig. 13(1)), the sites it can interact with are located exclusively inside the 'imprinted' cavities which swell preferentially facilitating insertion. For non-covalent systems, on the other hand, (Fig. 13(2)), owing to the introduction of an excess of functionalised monomer, three-quarters of the sites are located outside cavities. Swelling occurs throughout the whole polymer and leads to irreversible deformation of the recognition sites. This process stresses the importance of using functionalised monomers that can interact in stoichiometric proportions with the template molecule. The capacity of MIPs for the guest is generally low (typical values are 0.1 mg per gram of MIP). The development of new polymeric systems involving for instance the use of new crosslinkers (trifunctional or more) has led to significant increases in capacity: some systems are now able to resolve up to 1 mg of a racemic mixture of peptides using one gram of MIP [101]. These values are of the same order of magnitude as those obtained with "classic" chiral stationary phases.

3.2
Specificity of Recognition

On reinsertion of the guest molecule or of molecules with a similar structure, how can the specificity of the imprinted sites for the guest molecule be deter-

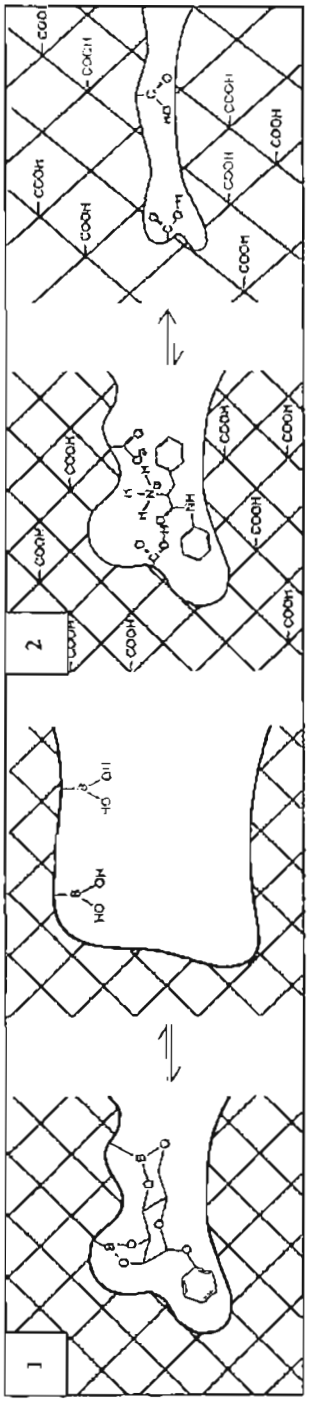


Fig. 13 Schematic representation of template molecule extraction and the resulting swelling of the network; (1) covalent system, 90–95% of the cavities are suitable for reinsertion; (2) non-covalent system, only 15% of the sites can be reused (adapted from [135])

mined on a macroscopic scale? In other words, how can the specificity of the recognition generate an easily identifiable signal? Owing in particular to the low capacity of the networks, this question is probably the most limiting for MIP applications. Two approaches have been considered to resolve this problem:

- Direct measurement of the interaction between the network and the guest molecule, when it is possible to quantify the variations of a property depending on these interactions. For instance, the introduction of fluorescent probes, either via the functionalised monomer or via the guest would allow accurate assessment of any change in the immediate environment of the probes [136]. The use of MIPs as recognition elements in sensors led to the development of this approach (see below).
- Indirect assessment of the interactions by observing the modifications brought about by the presence of the MIP on an element external to the network. For instance, using a MIP as a chromatographic stationary phase can provide a large amount of information about the network (capacity, specificity, association constants, etc.) by analysing the composition of the solution at the chromatograph outlet over time [114]. Similarly, for tests run in static conditions (batch rebinding) where the MIP is placed in a solution of known composition, the appearance or disappearance of molecules in the supernatant can be evaluated by isotopic labelling [31a] or spectroscopic analysis [30b].

Irrespective of how the problem is resolved, to evaluate accurately the contribution made by the molecular imprinting technique, the results obtained with the MIP should be viewed with respect to results obtained using a network with the same chemical composition synthesised in the same conditions but in the absence of the template molecule.

Remarks on the origin of the recognition process: Studies have shown in some instances that the position of the functions in the cavity was the major factor in the recognition process, the shape of the cavity as a whole simply improving selectivity [78a]. In a chiral context, experiments have shown that the chirality of the recognition sites caused, on a larger scale, chirality in the MIP polymer structure [137].

3.3
Lifetime of the MIPs

MIPs generally present good physical stability (mechanical resistance to high pressures and temperatures) [138] and good chemical stability (resistance to bases, acids, organic solvents and metal ions) [67, 138]. They can be reused over a hundred times and be stored at room temperature without losing their recognition specificity [50]. These elements give MIPs numerous advantages over their protein counterparts.

4
Main Uses of Imprinted Polymers

MIPs find uses in four main types of application: separation of molecules, preparation of antibody analogues, recognition elements for sensors, stereoselective reactions and catalysis.

4.1
Separation of Molecules

Imprinted materials can be used as stationary phase in affinity chromatography, especially for the separation of enantiomers. Compared to standard chiral stationary phases, these materials present the advantage of being synthesised "to order" for a given enantiomerically pure molecule. These chromatographic materials have allowed the separation of numerous compounds such as naproxene (anti-inflammatory) [139], timolol (beta-blocker) [50] and nicotine [140].

Separation performance can be expressed by the separation factor α, which is only dependent on the retention times of the two enantiomers, or by the resolution factor R_s which also takes into account the breadth of the chromatographic peaks. The higher these factors are, the better separation is. Remarkable selectivities have been obtained, for instance with an enantiomeric mixture of the dipeptide *N*-acetyl-Trp-Phe-O-Me (non-covalent system, R_s=17.8) [141] and

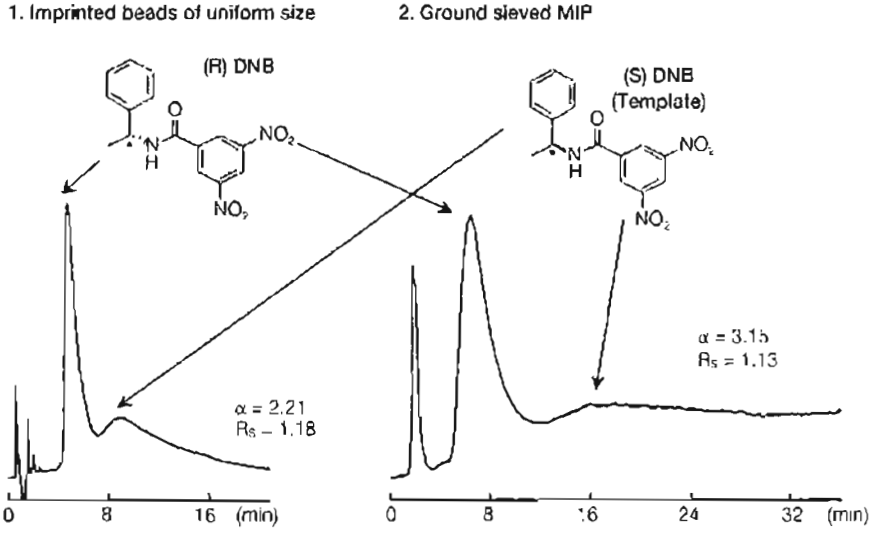


Fig. 14 Chromatographic separation of the two enantiomers of *N*-(3,5-dinitrobenzoyl)-α-methylbenzylamine (DNB) on networks imprinted around (S)-DNB. Effect of the mode of preparation on the chromatographic properties: (1) imprinted beads of uniform size (Fig. 12(2)); (2) ground graded MIP (Fig. 12(1)) (from [114])

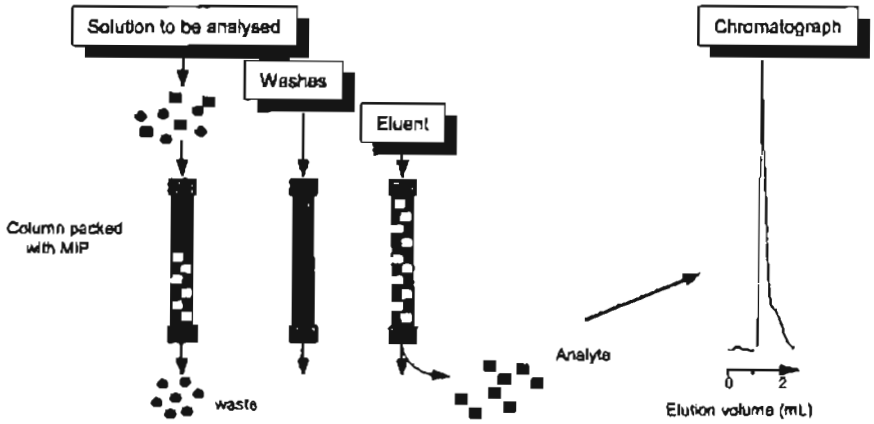


Fig. 15 Imprinted networks used for solid extraction. Explanatory schema

for a racemic mixture of phenyl-α-mannopyranoside (covalent system R_s=4.3) [142]. For example, Fig. 14 reports chromatograms obtained for the resolution of a racemic mixture of *N*-(3,5-dinitrobenzoyl)-α-methylbenzylamine obtained using the two materials of different morphologies presented in Fig. 12 [114].

The higher value of the separation factor α obtained with particles prepared by bulk polymerization can be explained by the weak template-MIP interactions involved (hydrogen bonding) when water is used as solvent, which decreases the strength of the interactions. In the same conditions of synthesis and use, the materials with a controlled structure ('beads') give a clearly higher resolution factor R_s.

These MIP were also successfully used for thin-layer chromatography [116] and capillary electrophoresis [105]. Extraction in the solid phase (Fig. 15) has also been extensively developed.

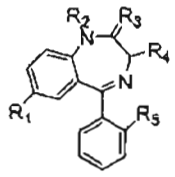
Fig. 16 Gas-phase chromatograms of extracts of human plasma; (1) after standard liquid-liquid extraction; (2) after solid-phase extraction with an imprinted polymer. Peak 1: guest molecule, R1=R2=CH_3, Peak 2: sameridine R1=CH_3, R2=C_2H_5, Peak 3: internal standard R1=R2=C_2H_5 (from [143])

In numerous fields, there is a great need for affinity matrices able to selectively extract and enrich analytes such as medical analysis, the food and drug industries and environmental applications. It has been demonstrated on many occasions that extraction on imprinted polymer can give better results than standard techniques such as liquid-liquid extraction, or extraction on C_{18} phase. Figure 16 gives the example of the extraction of sameridine, an analgesic, from human serum [143].

4.2 Preparation of Antibody Analogues

Recent studies have shown the efficiency of antibodies and artificial receptors prepared by the molecular imprinting technique and demonstrated the possibility of their use in therapeutic trials [102, 144]. Materials imprinted around diazepam (tranquilliser) and theophylline (broncho-dilator) templates have been found to have selectivities comparable to those of monoclonal antibodies and almost nil cross-reactions with related substances (Fig. 17) [102].

Receptors for morphine and for leu-enkephaline, efficient not only in organic media but also in aqueous solution, have also been synthesised [92]. Compared to their biological counterparts, imprinted networks present the advantage of being much more stable (chemically and physically), easy to synthesise and having a larger choice of template molecules.



	R_1	R_2	R_3	R_4	R_5
Diazepam	Cl	Me	O	H	H
Desmethyldiazepam	Cl	H	O	H	H
Clonazepam	NO_2	H	O	H	Cl
Lorazepam	Cl	H	O	OH	Cl
Alprazolam	Cl			H	H

Ligand in competition	Cross-reactivity (%)	
	MIP	Antibody
Diazepam	100	100
Desmethyldiazepam	27	32
Clonazepam	9	5
Lorazepam	4	1
Alprazolam	2	1

Fig. 17 Cross-reactivities of various benzodiazepines for the adsorption of ^{3}H-diazepam on the imprinted network. These reactivities are expressed as the molar ratio diazepam/competing drug inhibiting 50% of the ^{3}H-diazepam binding (adapted from [102])

4.3 Sensors

One of the most promising applications of imprinted networks is their use as recognition elements in sensors [145]. A reminder of the principle of action of these sensors is given in Fig. 18.

The MIP is in contact with a transducer which converts the chemical or physical signal obtained on adsorption of the analyte into an easily quantifiable signal. Various principles of transduction have been used: we can mention ellipsometry [146], electric capacity [115], conductimetry [147], piezoelectric microgravimetry [148], evanescent wave IR [149], fluorescence [136], amperometry [150], voltammetry [151] and pH [152]. The imprinted materials allow the highly specific detection of even very dilute molecules in mixtures that can be complex and in extreme conditions (e.g. high temperature). They are therefore used or have been considered for use on ions, molecules with therapeutic properties, combat gases (sarin and soman) [153], etc. Here again, their great stability gives them a real advantage over the biomolecules commonly used.

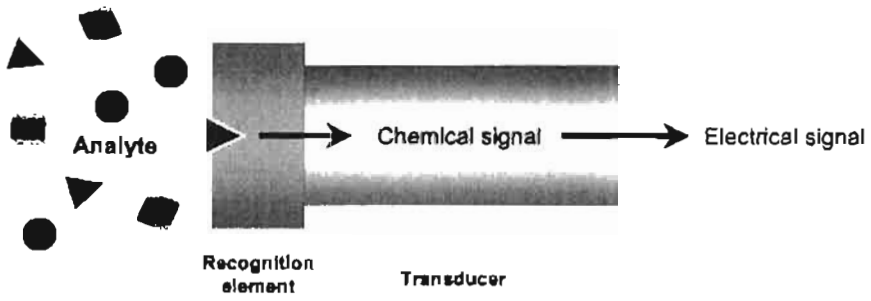


Fig. 18 Schematic representation of a sensor

4.4 Stereoselective Reactions and Catalysis

Reactions catalysed by enzymes are stereo- and regioselective. Abzymes and catalytic antibodies [154] constitute a first approach in mimicking such systems, but they are difficult to use.

Numerous research groups have attempted to make use of the selectivity of MIPs to prepare enzyme analogues with a catalytic activity [71, 155–170]. One strategy was to prepare a MIP around a template molecule with a structure similar to that of the substrate. The functional groups that play a catalytic role in the imprinted site are judiciously placed, by interaction with the functional groups on the guest molecule. An example of this strategy, represented in Fig. 19(2) [171, 172] concerns the catalysis of the dehydrofluorination of an

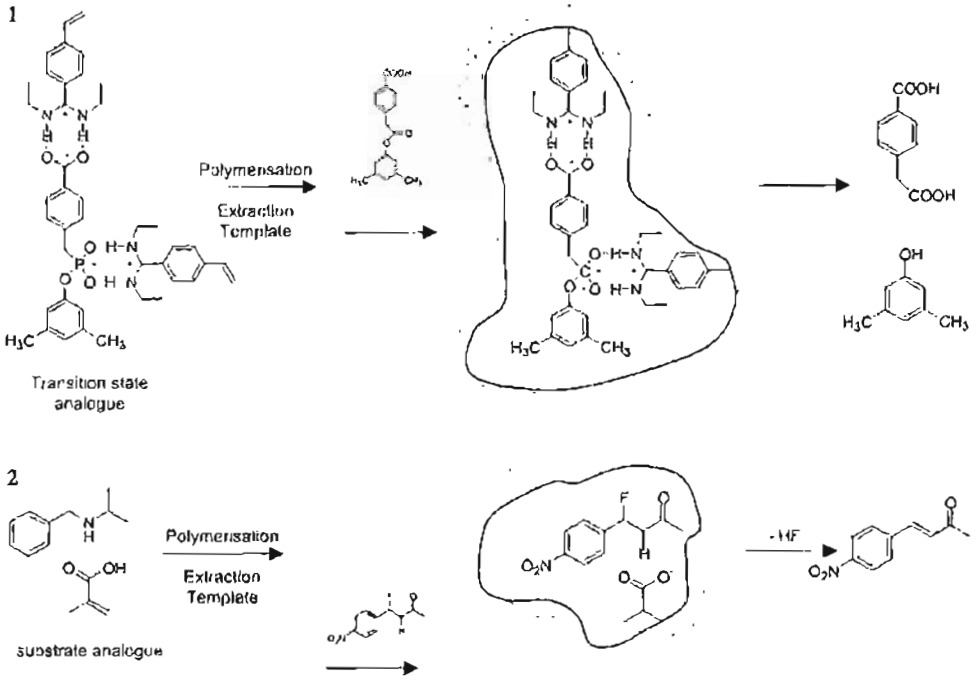


Fig. 19 MIPs used in catalysis: (1) ester hydrolysis (adapted from [156]); (2) dehydrofluorination (adapted from [171])

α-fluoroketone. The carboxylic catalytic group, placed in a favourable position by interaction with an amine function, catalyses the reaction very efficiently: the substrate, which is introduced afterwards, reacts 600 times faster than when the reaction occurs in solution. This value is close to that obtained with catalytic antibodies (1600 times faster) [171].

The most widely used strategy involves the synthesis of the network around a structural analogue of the transition state of the reaction. The imprinted sites then correspond to the conformation of the substrates in the transition state. For ester hydrolysis this state can, for instance, be simulated by a phosphonate derivative as template [156, 167]. An imprinted network with an esterase-type catalytic activity can then be obtained. For the MIP represented in Fig. 19(1), the reaction rate is increased 100-fold with respect to the reaction without catalyst and kinetics of the Michaelis-Menten type, as well as inhibition by an analogue of the transition state are observed [156].

Other reactions catalysed by MIPs have been described, including Diels-Alder type reactions [168, 170], aldol condensations [173] and isomerisation of benzisoxazoles [174], etc.

Apart from having a simple catalytic role, MIPs can be used in stereoselective [175, 182] and/or regioselective reactions [183]. In the example presented

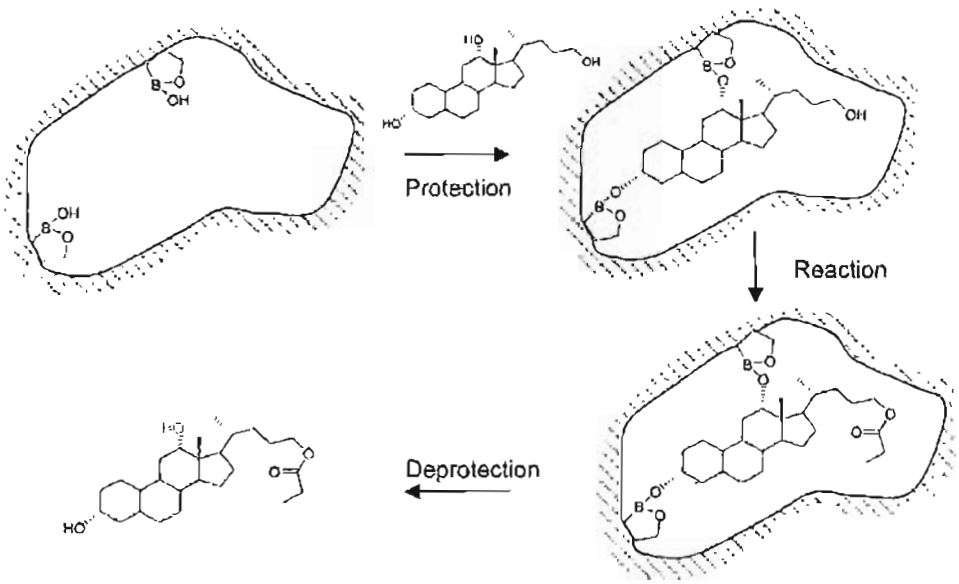


Fig. 20 Example of a regioselective reaction: the MIP enables acetylation on a specific hydroxyl group of the steroid while the other free hydroxyls are masked (adapted from [184])

in Fig. 20, a steroid with three free hydroxyl groups can be selectively acetylated on one of the hydroxyls, the others being masked by interactions with the imprinted network [184].

Finally, note that it has been proposed to modify enzymes using the principles of the molecular imprinting technique to modulate their action [185].

The catalytic activity of molecularly imprinted networks is still well below that of enzyme systems. However, even though imprinted materials cannot as yet compete with their biological counterparts, their high chemical stability and the possibility to use them in organic phases give them a promising future.

5
Summary and Outlook

The technique of molecular imprinting covers a wide range of applications. Compared to alternative techniques (involving biomolecules, abzymes, etc.), it presents a number of advantages: cost effectiveness, mechanical, thermal and chemical stability, long lifetime. As illustrated by the numerous patent applications, the first industrial uses for these materials have already been considered [186]. However, a certain number of factors limit the development of these materials.

In systems based on non-covalent interactions, a large excess of funtionalised monomer is introduced to favour the formation of the complex. The result is a random distribution of moieties liable to interact in addition to the active sites. This leads to a very heterogeneous population of recognition sites, lowering the performance of the imprinted networks (low capacity, reduced specificity, etc.). To overcome this, new monomers with a higher affinity for the guest molecule are being developed [59, 86].

In addition, the transposition of MIPs to aqueous media poses a certain number of problems related to the different types of interactions involved in this type of medium [134]. Similarly, for use in catalysis, much progress is yet to be made before industrial application can be considered [155].

Most of the drawbacks in MIPs have been linked to the fact that a large amount of crosslinker is needed (usually around 80–90%) to restrict distortion of the polymer backbone [17, 187]. The resulting stiffness of the network hinders the extraction and reinsertion of the template in the imprinted cavities and drastically decreases the capacity of the material [17]. Various 'surface imprinted' materials [117–122] have been reported to solve some of these problems but their capacities are very low.

Some promising materials have been proposed that have a certain rigidity maintaining the integrity of the recognition sites while remaining sufficiently flexible to enhance transfer of molecules and optimise the host-guest interactions.

These apparently antagonistic properties can however co-exist as shown by the way in which cell membranes operate (Fig. 1). The membranes occur in the form of a closed surface composed of a liquid film bathing in another immiscible liquid. They are essentially composed of a phospholipid bilayer integrating cholesterol, proteins and polysaccharides.

The hydrophobic alkyl chains are directed towards the interior of the film, whereas the hydrophilic poles cover the two faces of the film. Phospholipids tend to orient their chains perpendicularly to the film forming an ordered 2-D liquid. The proteins that are inserted into the bilayer themselves have regions that are more hydrophobic or more hydrophilic depending on the amino acids of the polypeptide chain. The hydrophobic parts are generally buried inside the bilayer while the hydrophilic parts protrude outwards. This membrane structure, although highly dynamic, remains coherent: the various molecules that make it up are in motion but the interactions that exist between them are sufficient to maintain the integrity of the structure. The supramolecular organisation is sufficient to maintain the recognition sites (especially on the proteins) in optimal positions to ensure that recognition is specific.

The use of systems based on supramolecular organisation therefore appears to be an interesting alternative in the technique of molecular imprinting. The integrity of the structure would not only be ensured, like in standard networks, by covalent chemical bonding but by the so-called "weak interactions" between the components of the network. It is in this light that polymer gels, 2-D films, and materials with a liquid crystal organisation have started to be developed.

5.1
Molecularly Imprinted Hydrogels

Hydrogels are cross-linked polymer networks that have the ability to absorb significant amounts of water. Their swelling behaviour can be modulated by external stimuli such as pH, temperature, ionic strength, electric field or concentration gradients. Tanaka and colleagues first obtained lightly crosslinked imprinted gels (less than 3 mol% crosslinker per mole monomer) that memorised their molecular conformation upon swelling and shrinking [188]. So, in a recent paper [189], Byrne and colleagues discussed the possibility of using a molecular imprinting technique in hydrogels to control the swelling behaviour of the material and so modulate their analyte binding ability (Fig. 21).

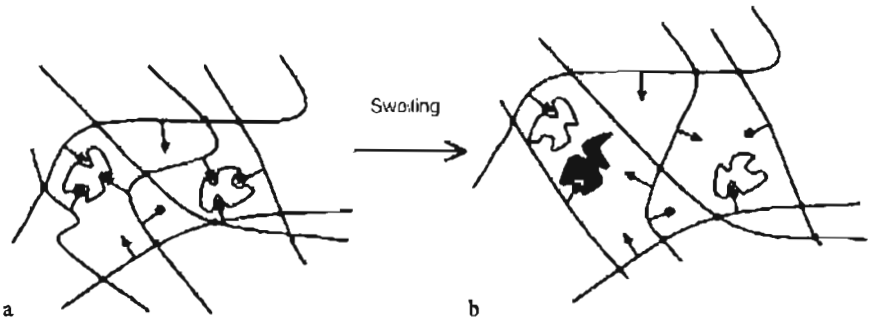


Fig. 21 Less crosslinked imprinted hydrogels: a rebinding of template; b swelling of the network leads to sites with varying affinity (adapted from [189])

5.2
Two-Dimensional Molecular Imprinting

Use has been made of lyotropic phases (such as micelles or vesicles) or Langmuir-Blodgett structures based on local 2-D organisation of molecules to form functionalised surfaces with a sharply defined molecular geometry. Two types of strategy have been applied to reach this aim:

- The simultaneous deposition of surfactant and template molecules, bound covalently or non-covalently, leading to the formation of a monolayer. [190, 28c]. This involves the formation of a deposit on a metal electrode (Fig. 22). The extraction of the template leaves a monolayer bearing sites allowing specific access to the electrode. The technique is well suited to the design of sensors.
- The formation of 2-D structures including functionalised parts. Mobility within the 2-D surfaces generated can optimise the positioning of the functionalised molecules with respect to the template. These structures can then be "frozen" by polymerising the vinylic surfactants used to form the layers [191]. In this way, Arnold et al. [87] prepared polymeric material function-

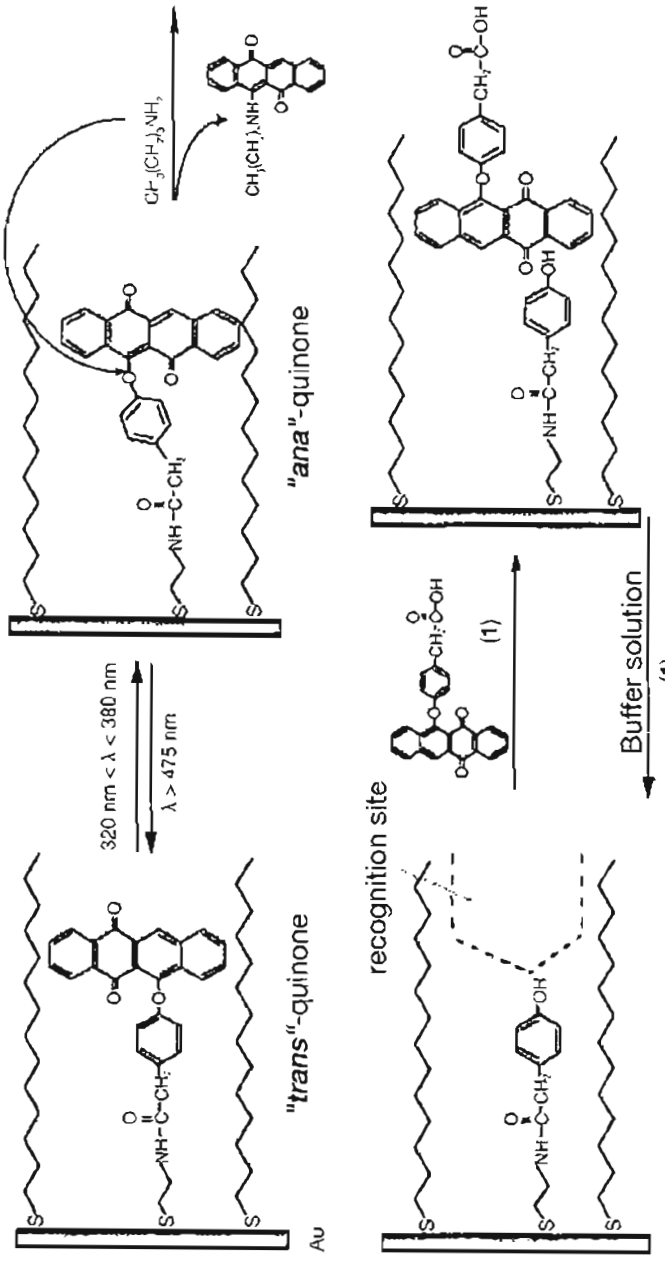


Fig. 22 Preparation of '*trans*'-quinone/$C_{14}H_{29}SH$ mixed monolayer electrode and the photoinduced formation of molecular recognition sites in the array. Uptake and release of '*trans*'-quinone to and from the sites (from [190])

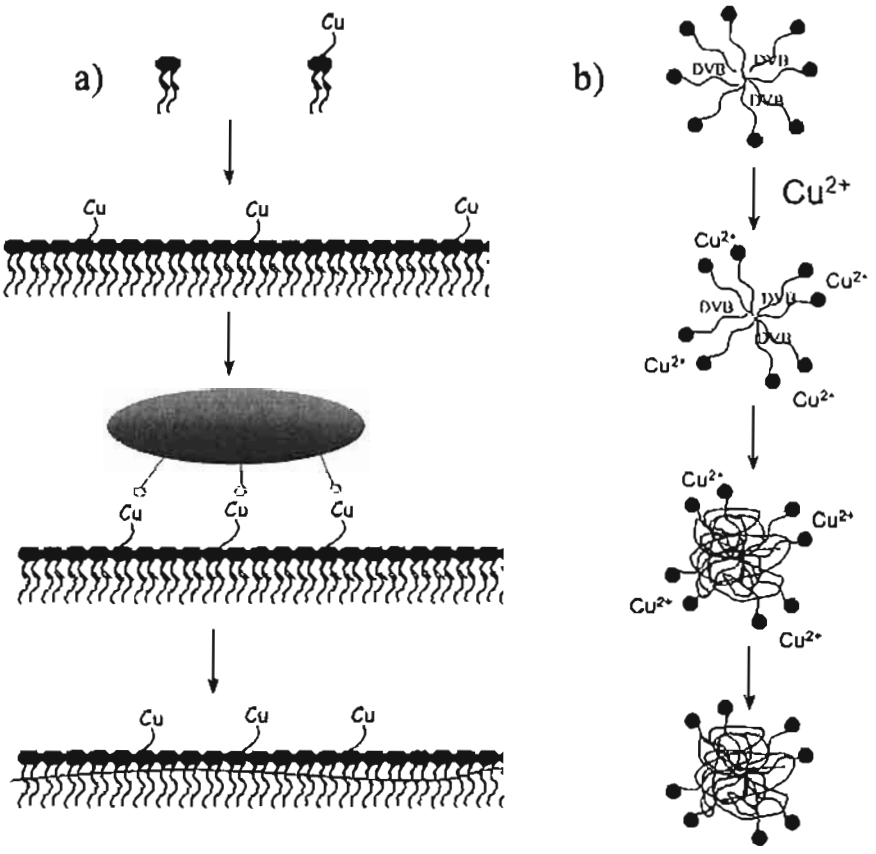


Fig. 23 a Schematic illustration of molecular imprinting of proteins on the surface of a metal-coordinating bilayer assembly (adapted from [87]). b Synthetic procedure for Cu(II) imprinted microspheres (adapted from [37, 124])

alised with metal ions to interact specifically with proteins (Fig. 23a). Likewise, superficially imprinted polymer beads were obtained from micellar structures [37, 124]. They presented good selectivity for the guest, which was the cupric ion (Fig. 23b).

In all cases, the imprinted surfaces generated in this way presented a higher affinity towards the template with respect to non-imprinted surfaces.

5.3 Liquid Crystalline Imprinted Materials

In order to soften the network while preserving the memory of the template, the use of liquid-crystal networks should be a useful tool. In such systems, the

interactions that develop between mesogenic substituents confers a stiffness to the network formed from non-covalent reversible interactions [192]. Moreover, any manifestations of the interaction between the polymer backbone and the mesogenic side-chains could be transmitted to the macroscopic level provided that chemical cross-links are introduced between the polymer backbones to form liquid crystal elastomers. Such behaviour was predicted by de Gennes [193] and subsequently a number of phenomena have been observed experimentally, including electrically-induced shape changes [194], strain-induced switching [195], transfer of chirality [196] and memory effects [197, 198]. The effect of cross-linking biases the structure towards the backbone configuration present at the time of network formation; any distortion of this configuration is opposed by the elasticity of the network. Consequently, such materials display a memory of both backbone anisotropy and (by virtue of coupling) side-chain orientation. As a result, in such liquid crystalline materials used as MIPs, the template can be extracted without losing the imprinted information even with low crosslinking ratios [197–200]. Moreover, the template would be easily extracted by use of a solvent that swells the network or by heating the network above the liquid-crystal/isotropic transition.

Liquid crystalline imprinted materials have been synthesized (Fig. 24) from polysiloxanes with mesogenic side-chains. In some of them, acetophenone was chosen as template and was covalently linked to the mesomorphous network via a ketal link [199]. In the others, the templates (carbobenzoxy-L-phenylalanine, 1,8-diaminonaphthalene or theophylline) were in interaction with the mesomorphous polymer via hydrogen bonding [200]. All the imprinted networks were obtained as dense membranes.

It was observed that a high proportion of template (10%) can be introduced without losing the mesomorphic order [199]. Moreover after template extraction, the polymorphism was quite different from that of the non-imprinted material, the variations depending on the structure of the template used and on its concentration [200]. The last point is the manifestation of a significant memory effect of the template, imprinted inside the mesomorphic structure. It arises from the interactions between template and the other parts of the network which can induce conformational constraints inside the networks during cross-linking. It occurs even though the amount of crosslinker is low (5%). Moreover, this point constitutes a means for the direct study of the imprint left in the network by the template.

Concerning molecular recognition properties, for all the materials synthesised, imprinted networks exhibit a much higher affinity towards the template than non-imprinted networks. For instance, in the case of 1,8-diaminonaphthalene used as the template [199], the amount of this molecule rebound by the materials in the mesomorphic state is reported in Fig. 25. It is obvious that the non-imprinted network exhibits significantly lower template uptake compared to the imprinted network. These results indicate that, in addition to the hydrogen bonding or electrostatic interactions between the functional groups of the polymers and template, microcavities corresponding to the shape of the

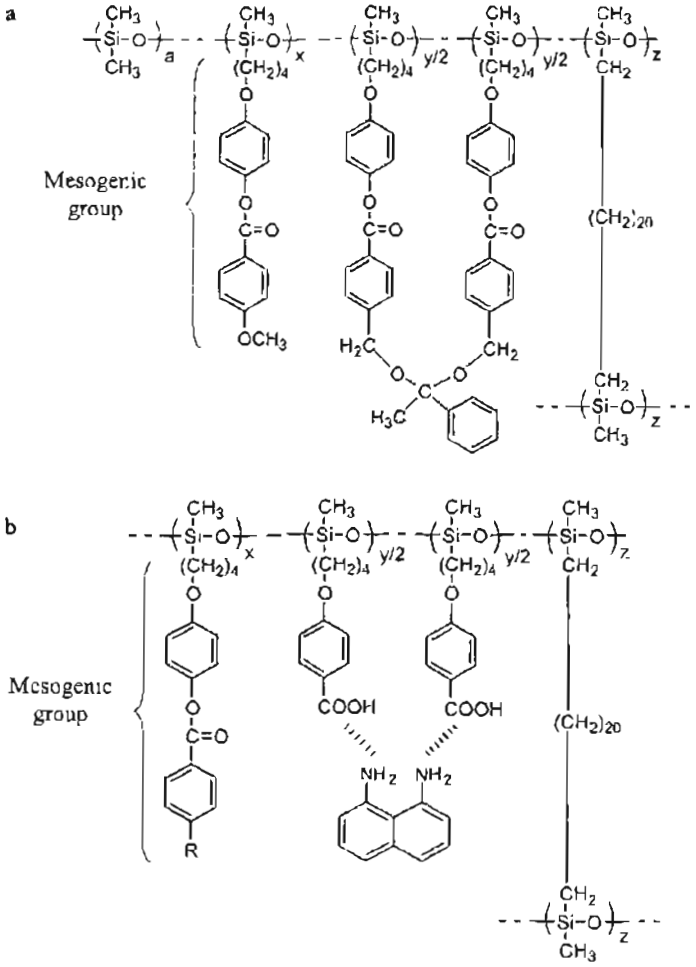


Fig. 24 Liquid crystalline materials imprinted via: a covalent linkages with acetophenone as template (adapted from [199]); b hydrogen bonding with 1,8-diaminonaphtalene as template (adapted from [200])

template are necessary for effective binding. Consequently, the polymer gains affinity for the template through the optimisation of molecular imprinting technology. Moreover, the network showed higher selectivity for the template than for a closely related compound (Fig. 25). Other experiments, obtained when theophylline or carbobenzoxy-L-phenylalanine were used as templates, have shown similar results [201].

On the other hand, the molecular trapping capacity of the networks (150 μmol/g of polymer) was shown to be much greater than that of most of the previously studied non-mesomorphous systems [197].

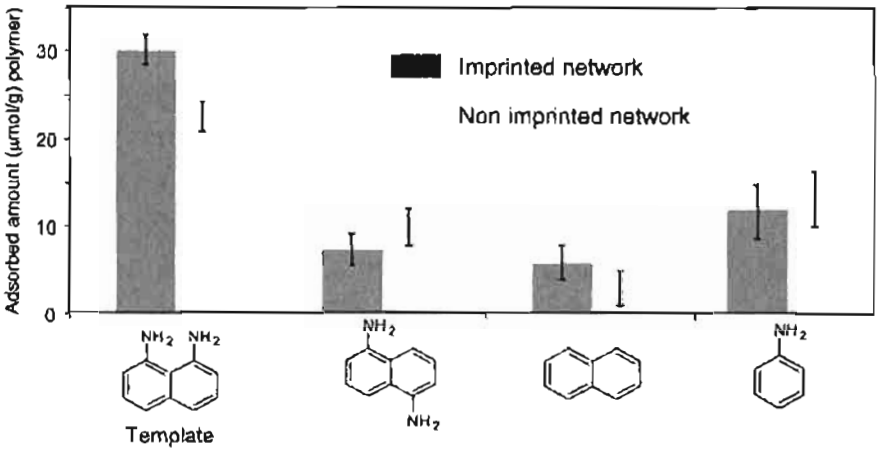


Fig. 25 Molecular recognition properties of a mesomorphous network imprinted around 1,8-diaminonaphtalene compared to a non-imprinted network (adapted from [201])

It is also possible to envisage exploiting other characteristics of liquid crystals to improve the recognition properties: the use of chiral mesophases to optimise the stereospecificity or modulate interactions between the recognition sites and the guest molecule by passing through the isotropic state, for example. These mesomorphic networks could also adapt to the geometry of the substrate to optimise the interactions occurring and thus enhance catalytic reactions.

6 Conclusion

The technique of molecular imprinting is currently in full development both from the point of view of design and study of the materials and of their actual domains of application. Widely used for the separation of molecules, it is now playing an increasingly active role in synthesis, in particular to generate stereospecific microreactors. The appearance of new materials involving supramolecular organisation appears promising in the improvement of recognition properties. Adapting the geometry of the cavities to the substrate and modulating it during the various phases of the process (formation, extraction, insertion), would then bring us another step closer to mimicking biological processes.

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