Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 804 (2004) 173–182
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Review

Molecularly imprinted polymers: synthesis and characterisation

Peter A.G. Cormack*, Amaia Zurutuza Elorza

Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow G1 1XL, UK

Abstract

This short review aims to present, in clear English, a summary of the principal synthetic considerations pertaining to good practice in the polymerisation aspects of molecular imprinting, and is primarily aimed at researchers familiar with molecular imprinting methods but with little or no prior experience in polymer synthesis. It is our hope that this will facilitate researchers to plan their own syntheses of molecular imprints in a more logical and structured fashion, and to begin to appreciate the limitations of the present synthetic approaches in this molecularly complex area, as well as the scope for rationally designing improved imprinted materials in the future.

Keywords: Reviews; Synthesis; Characterisation; Molecularly imprinted polymers

Contents

* Corresponding author. Tel.: +44-141-5484246.
E-mail address: peter.cormack@strath.ac.uk (P.A.G. Cormack).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.02.013

1. Introduction

From Glasgow to Timbuktu, scientists are replicating, adapting and evolving synthetic methods for the production of molecularly imprinted polymers in their own laboratories, in order to study and exploit the exquisite molecular recognition properties of these extraordinary materials for their own ends. Remarkably, such syntheses are just as likely to be carried out by a biochemist or an analytical chemist as by a synthetic polymer chemist. The inference that can be drawn from this observation is that syntheses of molecularly imprinted polymers need not necessarily be practically demanding, and indeed can even be accomplished in non-specialist laboratories with non-specialist equipment by researchers with little or no formal training in polymer synthesis. If this observation were made in isolation, one could easily be led to grossly underestimate the molecular complexity of molecular imprinting processes. Conversely, one is just as likely to hear talk of the "black art" of molecular imprinting. This implies complexity, serves to shroud molecular imprinting in mystery, fosters myths, tends to encourage researchers to cleave to accepted wisdom and, if anything, stifles innovation. The reality lies somewhere in between these two extremes; whilst it is true that the synthesis of molecularly imprinted polymers is an activity involving multiple (often inter-dependent) variables, a good understanding of the fundamentals of chemical equilibria, molecular recognition theory, thermodynamics and polymer chemistry, backed up with (increasingly) powerful analytical tools, helps to dispel these myths.

This short review aims to present, in clear English, a summary of the principal synthetic considerations pertaining to good practice in the polymerisation aspects of molecular imprinting, and is primarily aimed at researchers familiar with molecular imprinting methods but with little or no prior experience in polymer synthesis. It is our hope that this will facilitate researchers to plan their own syntheses of molecular imprints in a more logical and structured fashion, and to begin to appreciate the limitations of the present synthetic approaches in this molecularly complex area, as well as the scope for rationally designing improved imprinted materials in the future. The discussion begins with a general treatment of free radical polymerisation processes, and thereafter explains how cross-linked macromolecules may also be prepared via such methods. Thereafter, consideration is given to a number of issues relating specifically to molecular imprinting. Finally, a brief summary of the methods that can be used for the chemical and morphological characterisation of cross-linked macromolecules is presented. For reasons of brevity and clarity, the discussion is confined to the synthesis of imprinted *organic* macromolecules. It is not the intention of this article to merely regurgitate information from seminal treatises, but to collate disparate information and to present it in a fresh, coherent and accessible fashion; the reader will be redirected to the leading references for more detailed information where relevant.

2. Polymer syntheses

2.1. Free radical polymerisation

Free radical (or chain growth) polymerisation is the most important synthetic method available today for the conversion of monomer into polymer, and is exploited widely in industry for the production, on a multi-tonne scale, of a number of commercially important plastics. Numerous vinyl monomers can be polymerised very effectively in excellent yields by free radical polymerisation methods, including ethylene, styrene and methyl methacrylate which are of particular industrial importance. Free radical polymerisations can be performed under mild reaction conditions (e.g. ambient temperatures and atmospheric pressures) in bulk or in solution, and are very tolerant of functional groups in the monomers and impurities in the system (e.g. water). It is for these reasons, as well as the fact that many vinyl monomers are available commercially at low cost, that free radical polymerisation is usually the method of choice for preparing molecularly imprinted polymers.

The mechanism of free radical polymerisation is characterised by three distinct stages: (1) initiation, (2) propagation, and (3) termination. Detailed descriptions of these three stages can be found in any good textbook on polymer science and will thus not be reiterated here [1], but it is worthwhile emphasising two points. First of all, in a typical free radical polymerisation the rate of propagation (chain growth) is usually much faster than the rate of initiation, such that as soon as a new polymer chain starts to grow it propagates to high molecular weight in a relatively short period of time (perhaps within a second or two) before it terminates. What this means is that high molecular weight product is present in the system even when the amount of monomer consumed is low. Second of all, the source of free radicals (the initiator) is normally active over the entire duration of the polymerisation, such that if one were able to take a snap-shot of the system at any given instant in time, one would observe the presence of unreacted monomer and initiator, propagating (growing) polymer chains and high molecular weight polymer chains that were terminated (dead).

Many chemical initiators with different chemical properties can be used as the radical source in free radical polymerisation. Normally they are used at low levels compared to the monomer, e.g. 1 wt.%, or 1 mol.% with respect to the total number of moles of polymerisable double bonds. The rate and mode of decomposition of an initiator to radicals can be triggered and controlled in a number of ways, including heat, light and by chemical/electrochemical means, depending upon its chemical nature. For example, the azo initiator azobisisobutyronitrile (AIBN) can be conveniently decomposed by photolysis (UV) or thermolysis to give stabilised, carbon-centred radicals capable of initiating the growth of a number of vinyl monomers. As an illustrative example of the use of AIBN, or indeed other initiators, to polymerise vinyl monomers, AIBN can polymerise methyl

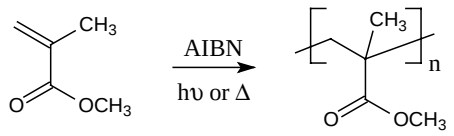


Fig. 1. Conversion of methyl methacrylate monomer by free radical polymerisation into poly(methyl methacrylate).

methacrylate under thermal or photochemical conditions to give poly(methyl methacrylate) (Fig. 1), i.e. Perspex^{TM}, a linear macromolecule that would be soluble in a thermodynamically compatible solvent such as toluene or tetrahydrofuran. Information on free radical polymerisation initiators is readily available from chemical suppliers, e.g. Wako Chemicals GmbH, and information on thermodynamically good solvents for any given macromolecule from a good literature source, such as the highly recommended Polymer Handbook [2].

2.2. Free radical copolymerisation

It is often highly desirable, not only in molecular imprinting circles, to simultaneously polymerise (copolymerise) two or more vinyl monomers within the same reaction vessel to give copolymers (as opposed to a homopolymer, which arises from the polymerisation of one single monomer). This allows products to be prepared with chemical properties distinct to the polymers obtained upon polymerising each monomer independently. For example, methyl methacrylate could be copolymerised with the more hydrophobic monomer butyl methacrylate to yield a copolymer product where, for any given polymer chain, there would be a statistical distribution of methyl methacrylate and butyl methacrylate units along the length of the polymer chain, and where the statistical distribution would be dependent upon the relative concentrations of the two monomers in the feed prior to polymerisation (Fig. 2a). The linear copolymer product, poly(methyl methacrylate-co-butyl methacrylate), would be soluble in a thermodynamically compatible solvent.

Particular care must be exercised in free radical copolymerisations to take account of the relative reactivities of the constituent monomers and to appreciate that all monomers are not consumed at the same rate, else the chemical composition of the copolymer products and the distribution of the monomer units within the copolymers may well be dramatically different to what one would predict on the basis of the monomer feed composition alone. As simple illustrative examples of this idea, certain pairs of monomers copolymerise to give specifically alternating copolymers (e.g. stilbene and maleic anhydride, Fig. 2b) irrespective of the monomer feed composition, whereas other pairs of monomers (e.g. maleimide and maleic anhydride) copolymerise inefficiently or not at all. It must also be pointed out that for any given pair of comonomers, the molecular composition of the resultant copolymer and the distribution of the monomer units within the copolymer are also dependent upon the relative monomer feed concentrations, and that this can vary with time.

Fortunately, the relative reactivities of many common monomers are known and have been tabulated, normally in the form of *reactivity ratios* for given pairs of monomers [2,3]. In a free radical copolymerisation of two vinyl monomers, A and B, where Monomer A is the ultimate monomer at the end of the propagating polymer chain, the rate constant for reaction of this polymer radical with Monomer A is given as k_{AA}, and the rate constant for reaction of the same polymer radical with Monomer B is given as k_{AB}. The reactivity ratio for Monomer A, r_A, with respect to Monomer B, is defined as the ratio of the two individual rate constants, i.e. $r_A = k_{AA}/k_{AB}$. By a similar analysis, the reactivity ratio for Monomer B, r_B, with respect to Monomer A, where Monomer B is the ultimate monomer at the end of the propagating polymer chain, is given as $r_B = k_{BB}/k_{BA}$. Whilst it is beyond the scope of the present article to enter into a thorough kinetic treatment of reactivity ratios such that the reader is once again directly towards a good polymer science textbook [1], it is nevertheless worthwhile to briefly outline how these values can be used to predict the likely outcome of a copolymerisation.

In a free radical copolymerisation of two vinyl monomers, A and B, the rate at which a given monomer is copolymerised depends upon the inherent reactivity of the monomer and

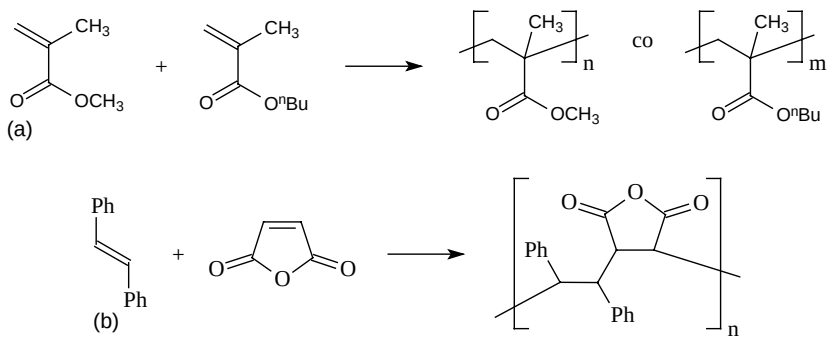


Fig. 2. Free radical copolymerisation of: (a) methyl methacrylate with *n*-butyl methacrylate, and (b) stilbene and maleic anhydride. Polymer (a) is a random copolymer whereas polymer (b) is a specifically alternating copolymer.

the radical derived from the same monomer, but also on how these reactivity values compare with the corresponding reactivity values for the second monomer. In practice, a rather complex picture emerges, especially as the rates of monomer propagation are also influenced by other experimental factors such as the reaction temperature and monomer concentration. Nevertheless, the reactivity ratios for monomers A and B, which are normally expressed as r_A and r_B, respectively, can be used to provide valuable insights into the likely outcome of a given copolymerisation involving A and B.

Reactivity ratio values normally lie in range 0–1, but can be much higher in some instances. A low reactivity ratio value implies low reactivity whereas a high value implies high reactivity. If the two monomers, when considered in combination, each have a moderate value (∼0.5, e.g. styrene and methyl methacrylate) then the copolymer formed will have a composition similar to, though not necessarily identical to, that of the monomer feed. If both monomers have a low value (∼0, e.g. stilbene and maleic anhydride) then copolymerisation will be slow and have a tendency to form specifically alternating copolymer. Finally, if one reactivity ratio value is high (e.g. styrene) whilst the other value is relatively low (∼0, e.g. vinyl acetate), then the tendency will be to consume one monomer preferentially near the beginning of the copolymerisation and the second monomer near the end of the copolymerisation, giving rise to an intimate mixture of homopolymer A and homopolymer B predominantly, rather than a copolymer.

The take home message as far as molecular imprinting and monomer reactivity is concerned, is that close attention must be paid to the relative reactivities of the monomers at the design stage of synthesis, as not all monomers are compatible with one another in copolymerisations. More specifically, they may not be incorporated statistically into the copolymer and, furthermore, their relative rates of incorporation may be dramatically different. Whilst the reactivity ratio values have not been tabulated for all monomers, approximations can be made and are useful (e.g. the tabulated values for methyl methacrylate are a useful guide to the values for ethylene glycol dimethacrylate).

2.3. Cross-linked polymers

All the polymerisations discussed hitherto involve the propagation (growth) of polymers derived from monomers with one single polymerisable vinyl group, hereafter referred to as mono-functional monomers. Mono-functional monomers normally polymerise to give linear macromolecules that are soluble in chemically compatible solvents. When multi-functional monomers, i.e. monomers bearing two or more polymerisable vinyl groups, are polymerised, either on their own or in combination with a comonomer or comonomers, then the outcome is quite different and this allows a number of non-linear polymer architectures of high commercial value to be prepared. These materials may be soluble or insoluble, and can be conveniently classified as branched macromolecules, microgels and macroscopic networks (Fig. 3) [4]. Multi-functional monomers are more commonly referred to as cross-linkers, and serve to chemically link two or more linear polymer chains.

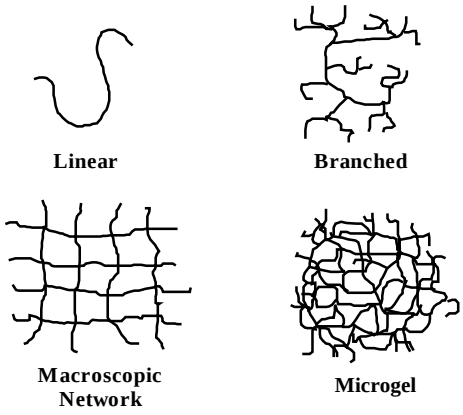


Fig. 3. Schematic representation showing polymers with different topologies: linear, branched, macroscopic network and microgel.

Branched polymers, microgels and macroscopic network polymers can often be prepared just as conveniently by free radical polymerisation as can linear polymers. To quote but one technologically relevant example, Merrifield resin [5] has found application in catalysis and solid-phase organic chemistry and can be readily prepared by the copolymerisation of styrene (as the mono-functional monomer) with divinylbenzene (as the cross-linker) to give a poly(styrene-co-divinylbenzene) macroscopic network, the chemical structure of which can be elaborated further if so desired (Fig. 4).

In the world of molecular imprinting, macroscopic polymer networks have been the non-linear polymers most widely synthesised and studied, as these tend to be insoluble species that lend rigidity and impart mechanical stability to an imprinted binding site. There have been some reports in the literature describing the imprinting of (soluble) microgels and linear macromolecules, but these are relatively few in number. For these reasons we will focus exclusively hereafter upon the synthesis of insoluble macroscopic network polymers, concentrating upon their distinct physical

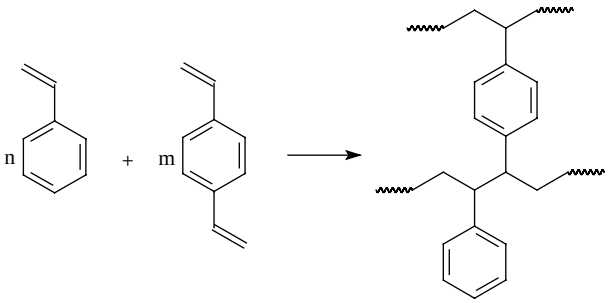


Fig. 4. Schematic representation of the cross-linked polymer network arising from the copolymerisation of styrene with *p*-divinylbenzene.

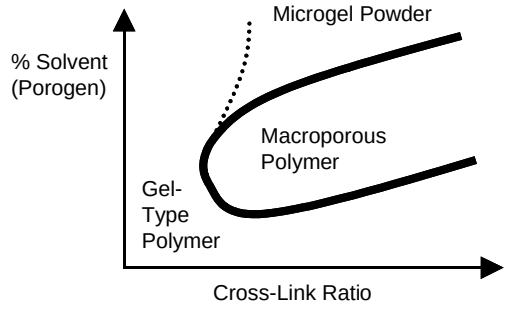


Fig. 5. Polymer pseudo-phase diagram clearly showing three distinct regions, i.e. gel-type polymers, macroporous polymers, and microgel powders.

properties and illustrating how these properties can be dramatically influenced by tailoring of the conditions under which they are prepared.

2.4. Gel-type polymers, macroporous polymers and microgel powders

In a copolymerisation involving a mono-functional monomer and a multi-functional monomer, two of the most important experiment parameters governing the physical nature of the product are the nominal cross-link ratio, defined as the percentage of cross-linker with respect to the total number of moles of monomer, and the volume (and nature) of the solvent in which the polymerisation is carried out. Upon collating the information obtained from numerous cross-linking polymerisations, where the levels of cross-linker and the volume of solvent have been systematically varied, it is possible to construct a pseudo-phase diagram (Fig. 5) which can be used to predict the physical nature of a polymer likely to arise from a given polymerisation [6]. For the purposes of the present review we will confine ourselves to a brief discussion of the three main regions apparent in the pseudo-phase diagram, i.e. gel-type polymers, macroporous polymers, and microgel powders. Whilst it is possible to make a number of generalisations, it is important to appreciate that the boundaries between the different polymer types in the pseudo-phase diagram are rather blurred, they are influenced by a number of factors besides the cross-link ratio and the volume of solvent (albeit to a lesser extent), and that there will inevitably be variations in behaviour upon moving from one polymer type to another, e.g. styrenic versus methacrylate.

At relatively low cross-linker ratios, e.g. <5%, or at higher cross-link ratios in the presence of low volumes of solvents which are thermodynamically compatible with the polymer network, then the situation can arise where phase separation of the polymer network does not occur during polymerisation. In such a case the product is a lightly solvated *gel-type polymer* which collapses upon drying to form an amorphous glassy gel-type polymer. Such materials typically have very low specific surface areas in the dry state on account of the polymer chains being in close molecular contact, swell significantly in thermodynamically good solvents, and have poor mechanical properties especially when the cross-link ratio is very low. Gel-type polymers have found few applications in molecular imprinting to date, not least because of their relatively poorer mechanical properties which makes them much less attractive for applications involving flow-through, e.g. HPLC.

At relatively higher cross-link ratios, and/or in the presence of higher volumes of solvent, the growing polymer matrix is able to phase separate from the polymerisation medium giving rise to macroporous polymers. The term “macroporous”, which can be used synonymously with the term “macroreticular”, is used to underline the fact that the polymers are porous, but is not meant to imply anything about the detailed morphology of the polymer, e.g. the average pore size or the pore size distribution. Macroporous polymers are characterised by having permanently porous structures, even in the dry state, and much higher specific surface areas than gel-type resins, with the knock-on effect being that even thermodynamically non-compatible solvents can access the pores. In addition, macroporous polymers are mechanically more robust than gel-type polymers on account of the higher levels of cross-linker present. It is for these reasons that macroporous polymers are generally preferred when one wishes to evolve effective molecularly imprinted polymers.

An interesting phenomenon is observed when the volume of solvent used is increased beyond that normally used to prepare macroporous polymers. Under these more dilute conditions the primary polymer particles that are formed, which normally fuse to form gel-type polymers or macroporous polymers under more concentrated conditions, remain in a non-aggregated state and are often directly recovered as powders. The primary particles are known as microgels and the products thus as microgel powders. In recent times microgel powders have become increasingly important as far as molecular imprinting is concerned, with recent developments in precipitation polymerisation enabling the routine synthesis of micrometer-sized spherical polymer particulates in good yields [7].

3. MIP syntheses

The challenge of designing and synthesising a molecularly imprinted polymer (MIP) can be a daunting prospect to the uninitiated practitioner, not least because of the sheer number of experimental variables involved, e.g. the nature and levels of template, functional monomer(s), cross-linker(s), solvent(s) and initiator, the method of initiation and the duration of polymerisation. Fortunately, a good number of “rules of thumb” have emerged in the literature that are helpful in this regard, however it is nevertheless useful to simultaneously bear in mind the basics of free radical polymerisation processes. In bringing this information together in a useful

fashion for synthetic purposes, it is constructive to highlight several of the more important considerations; whilst some of these synthetic considerations may be patently obvious, it is remarkable how often such things are often glossed over or indeed completely over-looked in the imprinting literature. In the following, attention is drawn to a number of factors pertaining to the template molecule and the selection of suitable functional monomers, cross-linkers, solvents, initiators and general polymerisation procedures.

3.1. Template

In all molecular imprinting processes the template is of central importance in that it directs the organisation of the functional groups pendent to the functional monomers. Unfortunately, and for a variety of reasons, not all templates are directly amenable to templating. In terms of compatibility with free radical polymerisation, templates should ideally be chemically inert under the polymerisation conditions, thus alternative imprinting strategies may have to be sought if the template can participate in radical reactions or is for any other reason unstable under the polymerisation conditions. The following are legitimate questions to ask of a template: (1) Does the template bear any polymerisable groups, (2) Does the template bear functionality that could potentially inhibit or retard a free radical polymerisation, e.g. a thiol group or a hydroquinone moiety, and (3) Will the template be stable at moderately elevated temperatures (e.g. at or around 60 °C if AIBN is being used as the chemical initiator) or upon exposure to UV irradiation.

3.2. Functional monomers

Functional monomers are responsible for the binding interactions in the imprinted binding sites and, for non-covalent molecular imprinting protocols, are normally used in excess relative to the number of moles of template to favour the formation of template, functional monomer assemblies (template to functional monomer ratios of 1:4 and upwards are rather common for non-covalent imprinting). It is clearly very important to match the functionality of the template with the functionality of the functional monomer in a complementary fashion (e.g. H-bond donor with H-bond acceptor) in order to maximise complex formation and thus the imprinting effect. When two or more functional monomers are used simultaneously in “cocktail” polymerisation [8] it is however also important to bear in mind the reactivity ratios of the monomers to ensure that copolymerisation is feasible (see earlier). In passing, it is also worth noting that complexation of a template by a functional monomer can also influence the reactivity of the monomer to some extent, as a result of pertubations to the electronics and/or the sterics of the monomer.

Scores of functional monomers with chemically diverse structures and polarities are commercially available and many more can be prepared by rational design. In Fig. 6 the chemical structures of a selection of the more important functional monomers are shown.

3.3. Cross-linkers

In an imprinted polymer the cross-linker fulfils three major functions. First of all, the cross-linker is important in controlling the morphology of the polymer matrix, whether it be gel-type, macroporous or a microgel powder. Secondly, it serves to stabilise the imprinted binding site. Finally, it imparts mechanical stability to the polymer matrix. Much has been written about the effect of the cross-linker on the molecular recognition behaviour of imprinted polymers, but from a polymerisation point of view, high cross-link ratios are generally preferred in order to access permanently porous (macroporous) materials and in order to be able to generate materials with adequate mechanical stability. Polymers with cross-link ratios in excess of 80% are often the norm.

For the same reason that one should match the reactivity ratios of functional monomers in a cocktail polymerisation to ensure smooth incorporation of the comonomers, the reactivity ratio of the cross-linker should ideally also be matched to that of the functional monomer(s). As discussed earlier, the reactivity ratios of cross-linkers may not be known in which case approximations can sometimes be made through studying the values of structural analogues. It should also be borne in mind that there may well be chemically distinct vinyl groups in multi-functional monomers with distinct reactivity ratios, i.e. different vinyl groups may be incorporated at differential rates into the polymer.

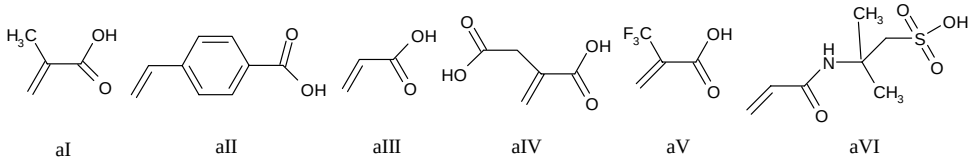
Quite a number of cross-linkers compatible with molecular imprinting are known, many of which are commercially available and a few of which are capable of simultaneously complexing with the template and thus acting as functional monomers. The chemical structures of several well-known cross-linkers are shown in Fig. 7.

3.4. Solvents (Porogens)

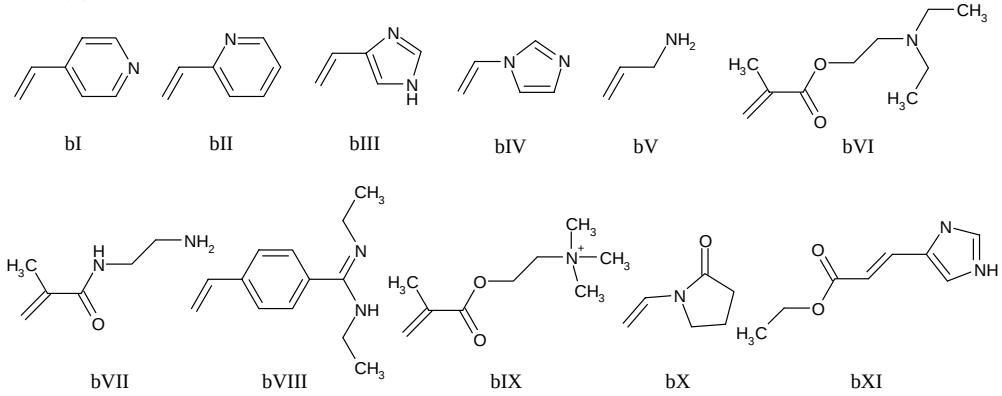
The solvent serves to bring all the components in the polymerisation, i.e. template, functional monomer(s), cross-linker and initiator into one phase. However, it serves a second important function in that it is also responsible for creating the pores in macroporous polymers. For this reason it is quite common to refer to the solvent as the “porogen”. When macroporous polymers are being prepared, the nature and the level of the porogen can be used to control the morphology and the total pore volume. More specifically, use of a thermodynamically good solvent tends to lead to polymers with well developed pore structures and high specific surface areas, use of a thermodynamically poor solvent leads to polymers with poorly developed pore structures and low specific surface areas. Increasing the volume of porogen increases the pore volume.

Besides its dual roles as a solvent and as a pore forming agent, the solvent in a non-covalent imprinting polymeri-

Acidic (a)



Basic (b)



Neutral (n)

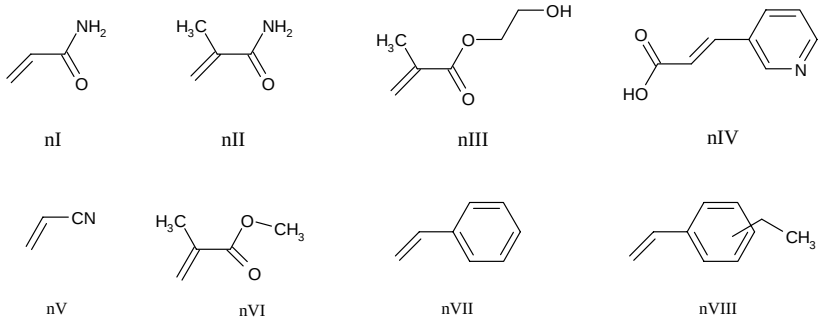


Fig. 6. Selection of monomers used in the non-covalent approach. **Acidic; aI:** methacrylic acid (MAA); **aII:** *p*-vinylbenzoic acid; **aIII:** acrylic acid (AA); **aIV:** itaconic acid; **aV:** 2-(trifluoromethyl)-acrylic acid (TFMAA); **aVI:** acrylamido-(2-methyl)-propane sulfonic acid (AMPSA). **Basic; bI:** 4-vinylpyridine (4-VP); **bII:** 2-vinylpyridine (2-VP); **bIII:** 4-(5)-vinylimidazole; **bIV:** 1-vinylimidazole; **bV:** allylamine; **bVI:** *N*,*N*′-diethyl aminoethyl methacrylamide (DEAEM), **bVII:** *N*-(2-aminethyl)-methacrylamide; **bVIII:** *N*,*N*′-diethyl-4-styrylamidine; **bIX:** *N*,*N*,*N*,-trimethyl aminoethylmethacrylate; **bX:** *N*-vinylpyrrolidone (NVP); **bXI:** urocanic ethyl ester. **Neutral; nI:** acrylamide; **nII:** methacrylamide; **nIII:** 2-hydroxyethyl methacrylate (2-HEMA); **nIV:** trans-3-(3-pyridyl)-acrylic acid; **nV:** acrylonitrile (AN); **nVI:** methyl methacrylate (MMA); **nVII:** styrene; **nVIII:** ethylstyrene.

sation must also be judiciously chosen such that it simultaneously maximises the likelihood of template, functional monomer complex formation. Normally, this implies that apolar, non-protic solvents, e.g. toluene, are preferred as such solvents stabilise hydrogen bonds, however if hydrophobic forces are being used to drive the complexation then water could well be the solvent of choice.

3.5. Initiators

In principle, any of the methods of initiation described earlier can be used to initiate free radical polymerisations in the presence of templates. However, there may well be drivers for selecting one over another arising from the system under study. For example, if the template were photochemically or thermally unstable then intiators that can be triggered photochemically and thermally, respectively, would not be attractive. Where complexation is driven by hydrogen bonding then lower polymerisation temperatures are preferred, and under such circumstances photochemically active initiators may well be preferred as these can operate efficiently at low temperature.

The chemical structures of selected polymerisation intiators are shown in Fig. 8.

3.6. General polymerisation procedures

The vast majority of monomers, especially liquid monomers, are normally supplied with polymerisation inhibitor present to suppress on-shelf degradation. Whilst it

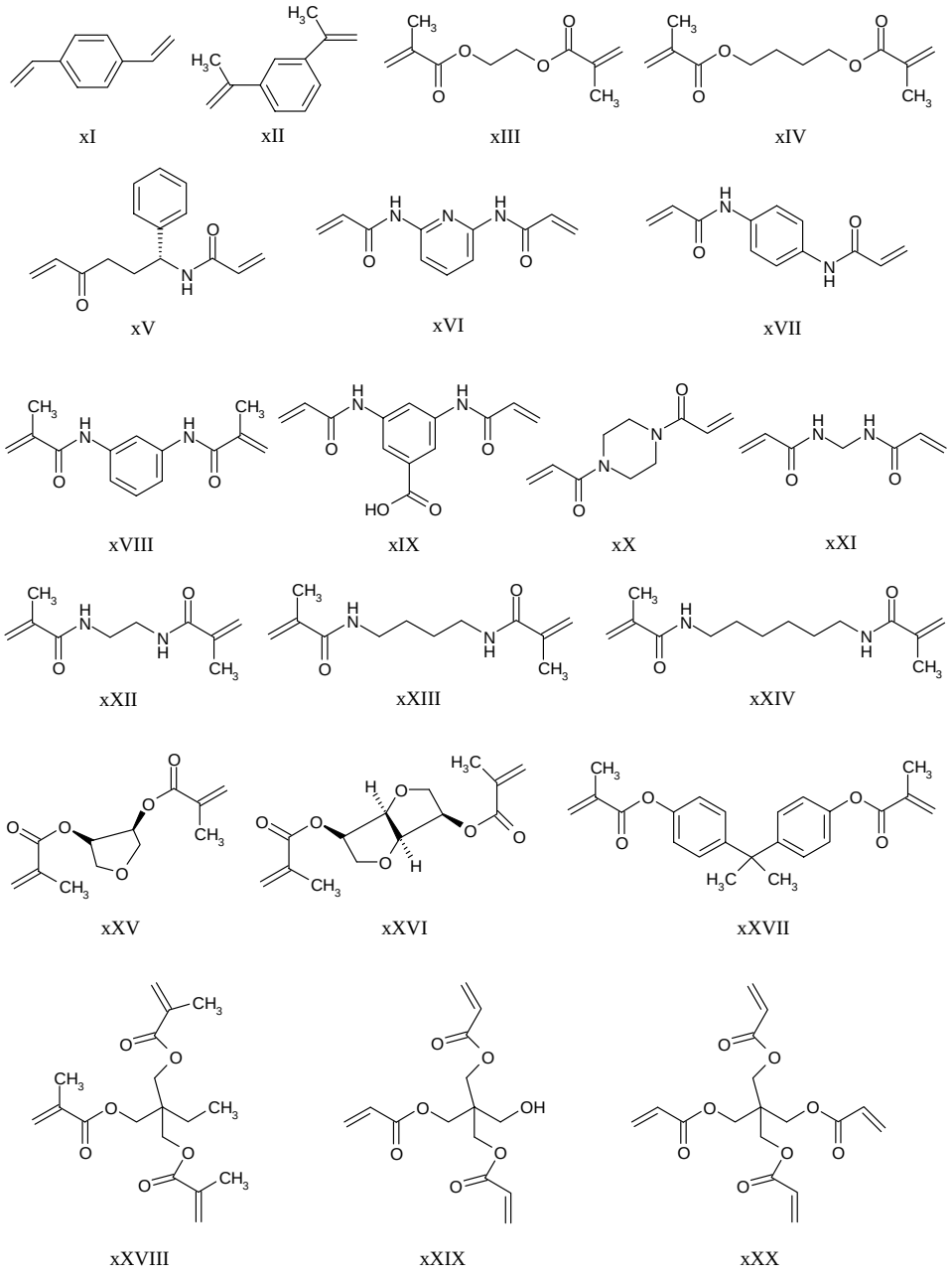


Fig. 7. Selection of cross-linkers used for molecular imprinting. **xI:** *p*-divinylbenzene (DVB); **xII:** 1,3-diisopropenyl benzene (DIP); **xIII:** ethylene glycol dimethacrylate (EGDMA); **xIV:** tetramethylene dimethacrylate (TDMA); **xV:** *N*,*O*-bisacryloyl-L-phenylalaninol; **xVI:** 2,6-bisacryloylamidopyridine; **xVII:** 1,4-phenylene diacrylamide; **xVIII:** *N*,*N*′-1,3-phenylenebis(2-methyl-2-propenamide) (PDBMP); **xIX:** 3,5-bisacrylamido benzoic acid; **xX:** 1,4-diacryloyl piperazine (DAP); **xXI:** *N*,*N*′-methylene bisacrylamide (MDAA); **xXII:** *N*,*N*′-ethylene bismethacrylamide; **xXIII:** *N*,*N*′-tetramethylene bismethacrylamide; **xXIV:** *N*,*N*′-hexamethylene bismethacrylamide; **xXV:** anhydroerythritol dimethacrylate; **xXVI:** 1,4;3,6-dianhydro-D-sorbitol-2,5-dimethacrylate; **xXVII:** isopropylenebis(1,4-phenylene) dimethacrylate; **xXVIII:** trimethylpropane trimethacrylate (TRIM); **xXIX:** pentaerythritol triacrylate (PETRA); **xXX:** pentaerythritol tetraacrylate (PETEA).

is certainly possible to polymerise monomer in the presence of inhibitor, especially when the levels of inhibitor are low and/or higher levels of polymerisation initiator are present, to ensure good batch-to-batch reproducibility of experiments, both in house and inter-laboratory, it is probably advisable to remove the polymerisation inhibitors from monomers, often in series with a second purification step, e.g. distillation. Such purifications are often straight-forward to perform, with many rigorous literature procedures being readily available [9].

Oxygen gas retards (slows down) free radical polymerisations, thus in order to maximise the rates of monomer propagation and to, once again, ensure good batch-to-batch reproducibility of polymerisations, removal of the dissolved oxygen from monomer solutions immediately prior to polymersation is advisable. Removal of dissolved oxygen can

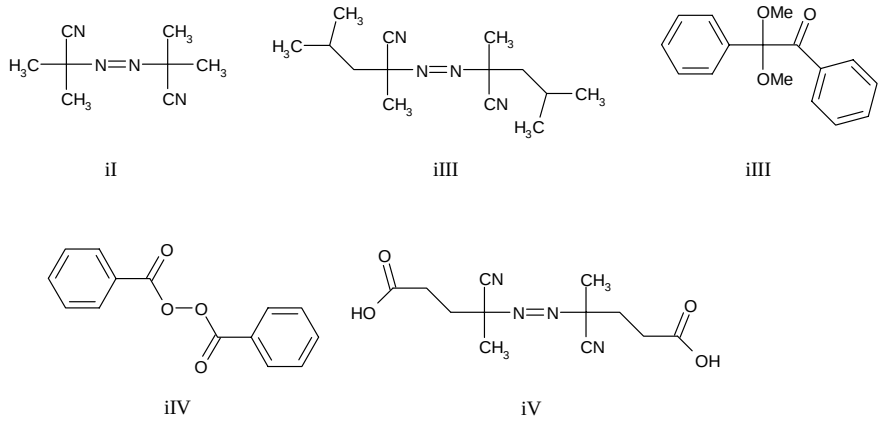


Fig. 8. Chemical structures of selected chemical initiators: **iI:** azobisisobutyronitrile (AIBN); **iII:** azobisdimethylvaleronitrile (ABDV); **iIII:** dimethylacetal of benzil; **iIV:** benzoylperoxide (BPO); **iV:** 4,4′-azo(4-cyanovaleric acid).

be achieved simply by ultrasonication or by sparging of the monomer solution by an inert gas, e.g. nitrogen or argon. If more rigorous degassing of monomer solutions is required, for whatever reason, then the method of freeze-pump-thaw comes into its own.

4. Polymer characterisation

Macroscopic network polymers are notoriously difficult to characterise largely on account of their insoluble, intractable nature. Imprinted polymers are no exception. A degree of characterisation is possible, [10] however, and one can distinguish between three levels of characterisation: (1) chemical characterisation, (2) morphological characterisation, and (3) characterisation of the molecular recognition behaviour. The molecular recognition aspect has been dealt with numerous times elsewhere, therefore we will restrict ourselves to a brief consideration of the chemical and morphological characterisation aspects, listing a few of the principle techniques available at the disposal of the analyst and the information that can be extracted.

4.1. Chemical characterisation

Given their insoluble, intractable nature, imprinted polymers are generally not amenable to characterisation methods involving the solution state, e.g. solution state NMR. Convenient analytical methods that can be used with solid samples to good effect include:

4.1.1. Elemental micro-analysis

Elemental micro-analysis can be used in a routine manner to measure the percentage by mass of carbon, hydrogen, nitrogen, chlorine, etc. within samples. When this technique is applied to the analysis of copolymers, the elemental composition information obtained can often be used to calculate the comonomer composition of the polymer; such calculations are particularly straight-forward to perform when one of the comonomers bears a heteroatom, e.g. nitrogen in the 4-vinylpyridine comonomer in poly(4-vinylpyridine-co-divinylbenzene). Unfortunately the method is not sufficiently sensitive to enable the detection of trace quantities of template remaining in molecularly imprinted polymers.

4.1.2. Fourier-transform infra-red spectroscopy (FTIR)

The FTIR spectra of imprinted polymers can be readily acquired (e.g. as a KBr disc or as a single bead or particle) and then applied in a similar fashion to elemental micro-analysis to extract quantitative information on the composition of the polymer. The method is of particular value when the different chemical environments in the sample (e.g. arising from the functional monomer and crosslinker in an imprinted polymer) give rise to well resolved, diagnostic signals. It is also possible to use FTIR to probe non-covalent interactions, e.g. hydrogen bonds, although the insensitivity of the technique sets limits on its utility in this regard.

4.1.3. Solid-state NMR

Solid-state NMR techniques circumvent the need to work in solution and therefore enable the NMR spectra of insoluble species to be acquired. For network polymers, insights into the different chemical environments present in the sample and information on the degree of chemical cure can be obtained. As far as molecular imprinting work is concerned, solid-state NMR has been relatively under-exploited to date, as has, for that matter, suspended state NMR.

4.2. Morphological characterisation

It is possible to probe the morphology of imprinted polymers in much the same way as one is able to do with most porous solids. Depending upon the method of analysis, useful information may be gleaned on the specific pore volumes,

pore sizes, pore size distributions and specific surface areas of the materials. Suitable analytical methods include:

4.2.1. Solvent uptake experiments

Macroporous polymers are permanently porous even in the dry state and solvent can be used to access the pore network. By measuring the amount of solvent uptaken by a polymer an estimate can be made of the specific pore volume (ml/g).

4.2.2. Nitrogen sorption porosimetry

Nitrogen sorption porosimetry involves a fixed mass of dry polymer being exposed to a gas (usually nitrogen) at a series of fixed pressures. By measuring the amount of gas sorbed as a function of pressure, sorption isotherms can be constructed from which, following application of theory (BET) and mathematical models, information on the specific surface area (m^2/g), specific pore volume (ml/g), average pore diameter and pore size distribution can be extracted. The method is particularly useful for analysing in detail medium-sized (meso-) and small (micro-) pores. (The IUPAC definitions of size as applied to pores are as follows: micropores <2 nm; 2 nm $<$ mesopores <50 nm; macropores >50 nm).

4.2.3. Mercury intrusion porosimetry

Mercury intrusion porosimetry involves mercury being forced, under pressure, into a fixed mass of dry polymer. The information that can be garnered from such experiments is similar to that which can be obtained from Nitrogen Sorption Porosimetry, although it is generally more sensitive at probing larger (macro-) pores.

4.2.4. Inverse size exclusion chromatography (ISEC)

In contrast to nitrogen sorption porosimetry and mercury intrusion porosimetry, which analyse polymers in the dry-state, ISEC enables the porous structure of polymers to be probed in the wet-state. This is perhaps significant as far as imprinted polymers are concerned because imprinted polymers find applications, more often than not, in the wet state. In a typical ISEC experiment, the porous solid is the stationary phase in a flow-through column set-up and the time taken for a series of linear soluble polymer standards of known molar mass to elute through the column measured at fixed flow-rate. Upon application of suitable mathematical models information on the pore structure of the polymer stationary phase can be extracted. In many respects ISEC can be viewed as being a complementary technique to nitrogen sorption porosimetry and mercury intrusion porosimetry, with the advantage being that it can operate in the wet-state.

4.2.5. Microscopy, e.g. SEM

Microscopy can be used in a variety of distinct ways to probe imprinted polymers on a variety of length scales. For instance, light microscopy can be used to verify the structural integrity of polymer beads whereas scanning electron microscopy (SEM) can often be used to image macropores.

5. Conclusions

The synthesis of molecularly imprinted polymers is a chemically complex pursuit and demands a good understanding of chemical equilibria, molecular recognition theory, thermodynamics and polymer chemistry in order to ensure a high level of success. Furthermore, optimisation of the imprinted products is made more difficult by the fact that there are many variables to consider, some or all of which can potentially impact upon the chemical, morphological and molecular recognition properties of the imprinted materials. Fortunately, in many instances it is possibly to rationally predict how changing any one such variable, e.g. the cross-link ratio, is likely to impact upon these properties. Furthermore, a clear understanding of the underlying principles of simple free radical polymerisation processes, especially when applied to macroscopic network polymers, provides a good basis upon which to make such predictions. Macroporous polymers, which have permanent pore structures even in the dry state, are particularly attractive matrices for imprinting not least because of their appealing mechanical properties. In turn, there are many ways in which the physico-chemical properties of macroporous polymers can be tailored to suit an intended application. Finally, the chemical, morphological and molecular recognition properties of molecularly imprinted polymers can be conveniently characterised by a complementary array of increasingly powerful analytical techniques.

References

[1] (a) F.W. Billmeyer, Textbook of Polymer Science, Wiley, New York, 1984;
(b) J.M.G. Cowie, Polymers: Chemistry and Physics of Modern Materials, Blackie Academic and Professional, London, 1991;
(c) G. Moad, D.H. Solomon, The Chemistry of Free Radical Polymerisation, Pergamon Press, Oxford, 1995;
(d) M.P. Stevens, Polymer Chemistry an Introduction, Oxford University Press, Oxford, 1999.
[2] J. Brandrup, E.H. Immergut, E.A. Grulke, in: Polymer Handbook, fourth ed., Wiley, New York, 1999.
[3] R.Z. Greenley, J. Macromol. Sci. Chem. A14 (1980) 445.
[4] W. Funke, Br. Polym. J. 21 (1989) 107.
[5] R.B. Merrifield, J. Am. Chem. Soc. 85 (1963) 2149.
[6] D.C. Sherrington, Chem. Commun. (1998) 2275.
[7] J.F. Wang, P.A.G. Cormack, D.C. Sherrington, E. Khoshdel, Angew. Chem. Int. Ed. Engl. 42 (2003) 5336.
[8] O. Ramström, L.I. Andersson, K. Mosbach, J. Org. Chem. 58 (1993) 7562.
[9] D.D. Perrin, W.L.F. Armarego, in: Purification of Laboratory Chemicals, fourth ed., Butterworth-Heinemann, Oxford, 1996.
[10] B. Sellergren, Molecularly Imprinted Polymers: Man-Made Mimics of Antibodies and Their Applications in Analytical Chemistry, Elsevier, Amsterdam, 2001.