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A Comparison of a Radical Polymerization vs ROMP Matrix for Molecular Imprinting

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ABSTRACT: We report a comparison of radical polymerization vs ROMP matrices in molecular imprinting technology. Although the radical polymerization method enjoys the greatest usage and applications, we found the ROMP method of polymerization not only improved the binding properties of the polymer but also increased the selectivity. The ROMP method creates the polymers much faster and uses mild conditions, moreover, the tolerance of Grubbs' catalyst to a large number of functional groups provides a wide range of molecules that can be used.

Introduction

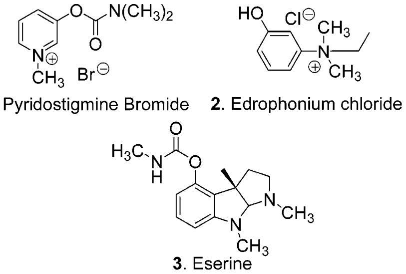
Molecularly imprinted polymers (MIPs), discovered about 35 years ago, represent a new class of materials that have artificially created receptor sites.[1] During the past decade, the development of assays, sensors, and membranes have been areas in which MIPs provide opportunities of advancements.[2] Molecular imprinting technology has recently developed into a viable approach for mimicking natural recognition entities, such as antibodies and biological receptors.[3] Polymer matrices with very high selectivity and stability can be obtained, finding applications in elevated temperature and pressure or reactions under acidic or basic conditions and in organic solvents.[4]

In this technology, recognition sites are created in a polymer matrix using a molecular template (print molecule) in a casting procedure. Radical polymerization is almost the exclusive method to prepare the MIP matrix; this polymerization functions well with a wide range of applications. Radical polymerization does have certain drawbacks because it cannot be used with some light/heat sensitive compounds and many unstable biomolecules like enzymes, hormones, and polypeptides.

There has been little work on ring-opening metathesis polymerization (ROMP) as an MIP matrix.[5] The recent discovery of well-defined transition metal catalysts for the metathetical polymerization of olefinic compounds makes available a wide range of unique materials.[6] The most effective catalysts have been those derived from ruthenium; these have been developed and promulgated by Grubbs. This paper reports on the comparison of ROMP vs radical polymerization matrices for the synthesis of MIPs. To our knowledge, this is the first comparison of these two matrices in MIPs.

Results and Discussion

We chose acetylcholine binding agents **1**−**3** for this study because of their biological interest, inherent positive charge, and inexpensive commercial availability, as shown in Figure 1. Eserine (**3**) has a mesomeric zwitterion or hyperconjugative form that leads to at least a partial positive charge on nitrogen.[7] The templates are polar and are able to create hydrogen bonds present in the MIP matrices.



1. Pyridostigmine Bromide **2**. Edrophonium chloride

3. Eserine

Figure 1. Acetylcholine binding agents.

For the radical polymerization[8] reactions, the template was allowed to form solution complexes prior to radical initiation with functional building blocks derived from methacrylic acid. These complexes were subsequently fixed using the cross-linking monomer, ethylene glycol dimethacrylate, into a rigid polymeric network, locking the complexes in position in the resulting material, as shown in Scheme 1. We used the usually reported template/monomer/cross-linking agent ratio of 1:4:16.[9] Thermal conditions with AIBN initiation were used because our templates were light sensitive. Mechanically grinding the polymer to a powder and removal of the print molecule by refluxing the polymer in ethanol/acetic acid then exposed the recognition sites. It was ensured that the print molecules were totally removed in each case by weighing the mass recovered after reflux and checking the NMR for of the recovered print molecule for authentication.

The ROMP matrices using Grubbs' catalyst provided a cross-linked polymer insoluble in methylene chloride in less than 1 min, while it took about 10 h for each radical polymerization to go to completion. Different template/monomer/cross-linking agent[10] ratios were tested, and it appeared that the best one, in terms of rate of polymerization and mechanical properties of the polymer, was 1:4:16, exactly the ratio used for the radical polymerization studies. Second generation Grubbs' catalyst proved to be the most efficient; it allowed not only a faster polymerization but also a higher degree of cross-linking.[6] After removal of the solvent, the remaining solid was ground to a fine powder, which was subsequently refluxed in ethanol to remove the template, as shown in Scheme 2. After filtration the powdered MIP was ready for selectivity tests.

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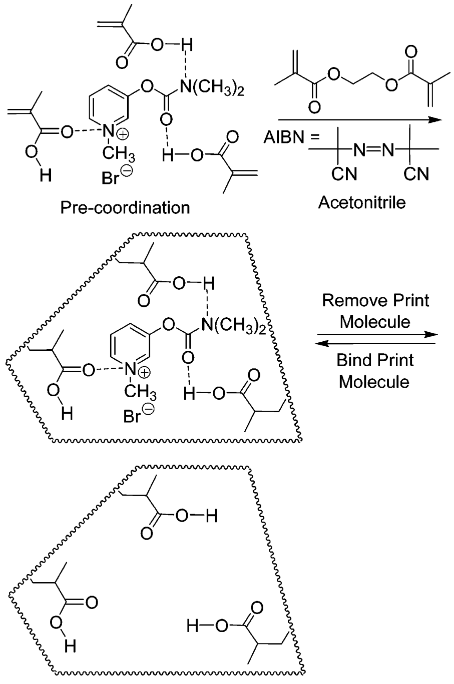
Scheme 1



Scheme 2

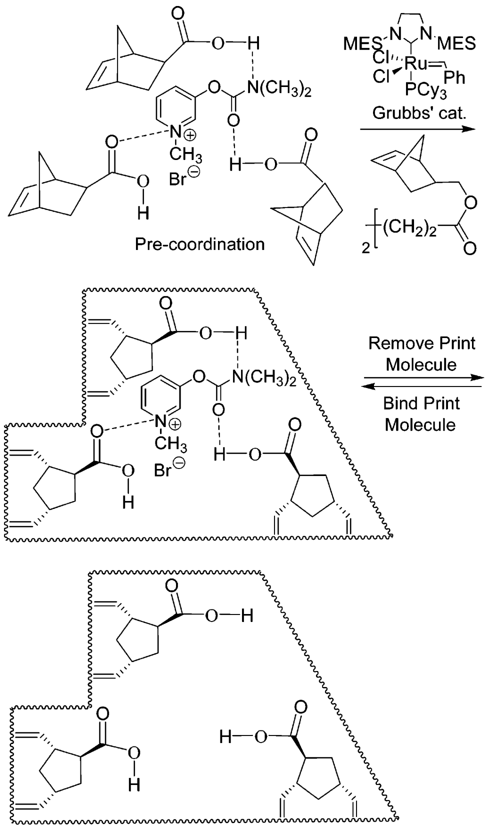


Chart 1. Radical Polymerization Matrix with Edrophonium Chloride as the Print Molecule

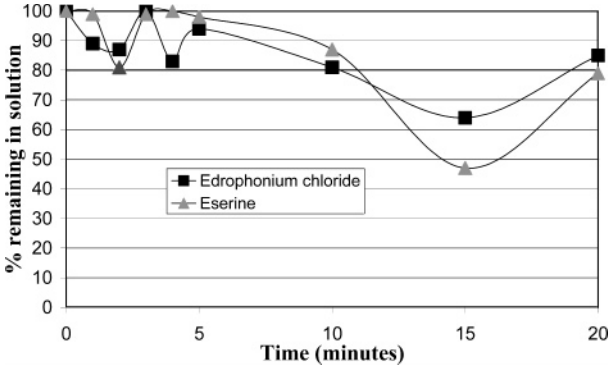
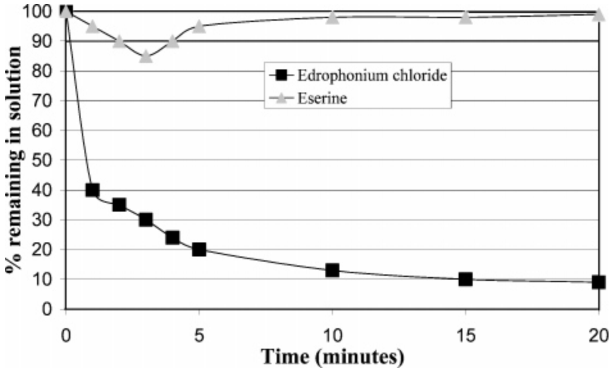


Chart 2. ROMP Matrix with Edrophonium Chloride as the Print Molecule



We used GC−MS methods to determine the competitive affinity of the polymer for two of the acetylcholine binding compounds, discussed above. Each polymer was suspended in a solution of the print molecule: either pyrostigmine bromide (**1**) or edrophonium bromide (**2**). Eserine (**3**) was selected as the compound competing for the sites on the MIP with either **1** or **2**. Aliquots were taken over time after after removing the polymer by filtration and the remaining solution was analyzed by GC/MS to determine its composition. From the starting amount of material and the amount present in each aliquot, we could quantitatively measure the percentage of binding for each compound to the polymer matrix. The percentage (%) of what remains in solution for both the template and the test molecule were calculated and plotting those against time allowed us to quantitatively compare the binding of each molecule. Comparing the selectivity properties of the radical polymerization vs ROMP matrices was then possible.

The first print molecule examined was edrophonium chloride (**2**), with its MIP formed under radical polymerization conditions. When eserine (**3**) and edrophonium chloride (**2**) molecules were in competition for the polymer binding sites in solution, there was little differentiation, as shown in Chart 1. The peaks and troughs indicate an exchange process; that is, the concentration of the test molecule and the templates kept changing over several minutes. However, the troughs provided by the polymer formed under free radical conditions were not selective enough to trap either molecule preferentially. The strongest attraction occurred at the trough at the 15 min mark leading to further dissociation after 20 min. It appears that over time the radical MIP became less selective. The templates and test molecules were not well-differentiated by the radical polymerization matrix.

Next, we examined the same print molecule, edrophonium chloride (**2**), with its MIP formed under ROMP conditions. When eserine and edrophonium chloride molecules were in competition for the polymer binding sites in solution, the selectivity was greatly enhanced and only the desired template molecules (edrophonium chloride) got trapped in the matrix, as shown in Chart 2. At 20 min, over 98% of the eserine remained in solution compared with only 9% of the edrophonium chloride. This result indicates that the MIP made by ROMP methodology gave more selective and better shaped cavities than the ones present in the radical-induced MIP.

Chart 3. Radical Polymerization Matrix with Pyridostigmine Bromide as the Print Molecule

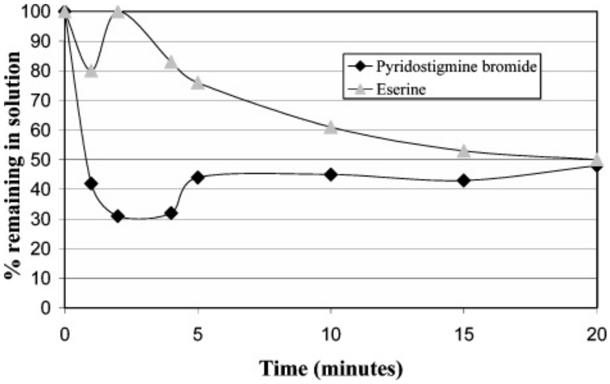
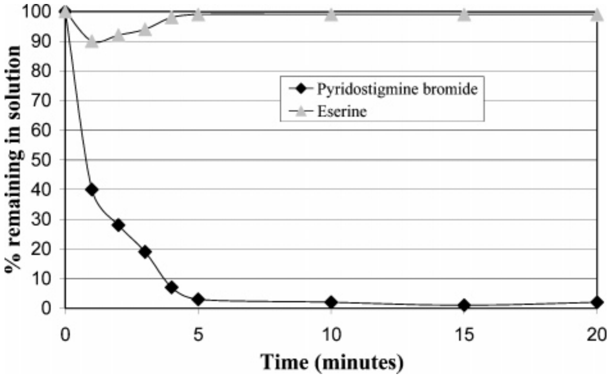


Chart 4. ROMP Matrix with Pyridostigmine Bromide as the Print Molecule



The second print molecule we studied was pyridostigmine bromide (**1**) which followed a behavior similar to edrophonium chloride. Once again, the radical polymerization polymer remained fairly unselective, as shown in Chart 3. With eserine and pyridostigmine bromide binding in competition, the polymer seemed unable to differentiate them. The pyridostigmine bromide appears to bind best early in the test at the 2−3 min mark, the bound less strongly as time progressed. However, with the ROMP polymer, the selectivity was greatly enhanced and only the print molecule pyrostigmine bromide was absorbed in the matrix (Chart 4).

In both cases, we were pleased to see that the "trapping" process of the template took less than 20 min. Note that over 98% of the eserine remained in solution while only about 3% of the pyridostigmine bromide remained in solution. The ROMP matrix appears to be much more selective in this case, as well.

Rather than having the print molecules in the cavities within the MIP matrix, they could simply attached on the surface of the polymer. Thus, the selectivity provided by the MIP would not come from the cavities but only from the nature of the interactions between the molecules and the surface of the polymer. To make sure that the molecules were not on the surface of the polymer (adsorption), an analysis was performed with blank polymers (no print molecule) for both the radical polymerization and ROMP MIPs. Each blank polymer was mixed with one solution of pyridostigmine bromide, edrophonium chloride or eserine of known weight (the conditions and quantities used in these control tests were identical to those used in the synthesis of the printed polymers). The mixture was stirred under argon and after 10 min, the suspension was filtered. The filtrate was concentrated and the percentage of mass recovered was calculated. The results are reported in Table 1. The control experiments showed that only a small quantity (average of 3.5%) of molecules was trapped by the blank polymers, meaning that the surface was not responsible for the high selectivities previously observed.

Table 1. Control Experiments

entry	MIP	compd	% recov	% abs
1	radical polymerization	**1**	98 ± 4	2 ± 4
2	radical polymerization	**2**	95 ± 1	5 ± 1
3	radical polymerization	**3**	96 ± 3	4 ± 3
4	ROMP	**1**	95 ± 1	5 ± 1
5	ROMP	**2**	98 ± 4	2 ± 4
6	ROMP	**3**	96 ± 3	4 ± 3

Conclusion

We report a comparison of ROMP vs radical polymerization matrices in molecular imprinting technology. The ROMP method of polymerization not only improved the binding properties of the polymer but also the selectivity. It is also worth noting that the comparison of the two polymers is not a perfect one. Different polymers and different starting compounds (methacrylic acid vs norbornenes) were used for radical polymerization vs ROMP matrices. It is also possible that eserine does not bind well to the ROMP polymers, too. As we compared them here, the ROMP method is faster and uses milder conditions. It is now possible to synthesize highly selective MIPs in a few minutes, not hours. The tolerance of Grubbs' catalyst to a large number of functional groups also provides a wide range of molecules to be used.

Experimental Section

General Procedure for Preparation of a Radical Polymerization Matrix. Pyridostigmine bromide (0.75 mmol, 195.8 mg) was added to a round-bottom flask, methacrylic acid (30.0 mmol, 0.28 mL) was poured and the mixture is allowed to stir for 10 min under argon. Ethylene dimethacrylate glycol (12.0 mmol, 2.25 mL), AIBN (0.17 mmol, 28 mg) and distilled acetonitrile (3.0 mL) were added. The mixture is refluxed for 8 h until no pyridostigmine bromide could be observed on TLC. The white solid obtained was then ground, suspended in ethanol and refluxed overnight to remove the template (pyridostigmine). The powder was dried under vacuum.

General Procedure for Preparation of a ROMP Matrix. Pyridostigmine bromide (0.75 mmol, 195.8 mg) was added to a round-bottom flask flushed with argon; dinorbornene cross-linking agent[8] and 5-norbornene-2-carboxylic acid (30.0 mmol, 0.28 mL) were dissolved in distilled methylene chloride and the mixture is allowed to stir for 10 min under Ar. Second generation Grubbs' catalyst[4] (2 mol %) was then added to the solution and a "gum-like" gel forms in 5 min. This material was then ground in a mortar, the powder was suspended in methylene chloride and ethylvinyl ether was added to remove any excess of monomers and Grubbs' catalyst. The solution was filtered and the powder was suspended in ethanol with acetic acid and refluxed overnight. The polymer was filtered out using a Buchner funnel and the material is allowed to dry.

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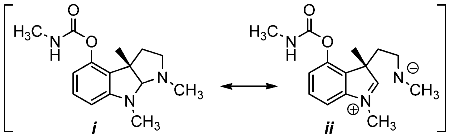
(7) The resonance structure ***ii*** shows the partial positive charge on nitrogen in eserine:



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(10) The dinorbornene cross-linking agent has a CAS Registry Number: 7359-19-5.

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