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Molecularly imprinted sol gel for ibuprofen: An analytical study of the factors influencing selectivity

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ABSTRACT

This paper describes the preparation and testing of a sol gel specific for the non-steroidal anti-inflammatory drug ibuprofen. Ibuprofen was selected as a model compound due to the fact that it contains a number of structural and functional analogues, in this case ketoprofen and naproxen. In order to study the specific criteria affecting selectivity in sol gels, three sol gels were prepared for ibuprofen utilising two and three functional silane systems. The relative rebinding of each of the three compounds to the sol gels was assessed by % recovery in solid phase extraction. The results of the experiments indicate that along with the functionality imparted to the sol gel by the development of template-monomer complexes a major determinant of selectivity is shape selective memory. The utilisation of a three monomer system affords the cavity recognition based on the formation of π–π stacking interactions, hydrogen bonding, van der Waals forces, electrostatic interactions and shape complementarity and minimises cross reactivity. In addition real sample analysis has been performed on urine samples containing ibuprofen and metabolites showing specific preconcentration.

1. Introduction

Molecular imprinting technology is now an established technique for the production of synthetic receptors. The mechanism of imprinting is based on the prearrangement of a template—functional monomer complex in a facilitating solvent (porogen). Applications of MIPs in analytical chemistry are diverse and include solid phase extraction [1–3], binding assays [4,5] and incorporation into biosensors [6,7].

Pauling [8], first proposed the concept of prearrangement of monomer units around a chemical species (in this case an antigen) in terms of explaining the production of antibodies in mammalian systems. The arrangement of these monomer units around the antigen was achieved by weak intermolecular interactions such as electrostatic interactions, van der Waals forces and by hydrogen bonding. Although abandoned as an immunological concept, the work of Pauling resurfaced in the studies of Dickey [9]. Dickey noted that a silica gel synthesised in the presence of methyl orange exhibited selective memory for this compound when rebinding experiments were performed in the presence of related compound structures (n-butyl, n-propyl and ethyl orange). This (early) process whereby nanostructured silica based solids exhibit selective molecular recognition is now referred to as the sol gel process. Imprinted sol gels are a rapidly developing area of synthetic receptor science. They have had application in sensor development [10], solid phase extraction or selective clean up [11] for analysis of the specific compounds propanolol [12], 2,4-dichlorophenoxyacetic acid [13], nafcillin [14], for selective discrimination of methylxanthines [15] and in protein/peptide recognition [16,17].

Sol gels are based on a silica backbone and inorganic-organic hybrid materials based on organically modified silicas (ormosils) offer an attractive alternative to molecularly imprinted polymers. Indeed imprinted sol gels possess, numerous significant advantages over MIPs, namely, ease of preparation, gelation at ambient temperatures (particularly important when preserving weak interactions). In addition to this sol gels exhibit significantly higher porosity and surface area than MIPs along with negligible swelling in organic solvents and good optical (transparent) properties [18].

Sol gel chemistry utilises mild acid- or base-catalysed conditions to achieve hydrolysis and condensation of numerous silane monomers. Gelation of the silane(s) such as tetraethyl orthosilicate (TEOS), 3-Aminopropyl triethoxysilane (APTES) and phenyltriethoxy silane (PTMOS) under aqueous conditions with alcohol (usually ethanol or ethoxyethanol) in the presence of a template molecule leads to the imprinted sol gel. The pH of the mixture will determine whether the dominant process is hydrolysis or condensation. At low pH, i.e. acid catalysed sol gels, condensation occurs at an enhanced pace in comparison to hydrolysis. The result of this being that polymer growth is favoured over cross-linking. The resultant acid catalysed gel is optically transparent with very small pores

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(2–50 nm) and high surface area (>200 m^2/g). Sol gels have significant potential in the development of thin films and layers. A sol gel film has been developed for the analysis of propanolol [12]. A direct comparison between the sol gel and the acrylic based molecularly imprinted polymer was performed. The findings were that the sol gels exhibited a lower total uptake of propanolol but significantly lower non-specific binding. Furthermore, the binding was found to be abrogated when aqueous solutions were replaced with organic solvents. Since it is known that sol gels do not exhibit the same degree of swelling as MIPs do, the loss of affinity may be due to shrinkage of sol gels. Sol gels in bulk form however have strong potential for use in solid phase extraction and recently these applications have been highlighted [11,19]. Given that swelling and changes in pH should not be as significant an issue as with MIPs, this means that the internal structure of the nanocavities within the sol gel should be maintained when loading in organic solvents or at lower pHs. Furthermore given that the nature of the functional interactions is non-directional in nature (hydrogen bonding, electrostatic, van der Waals) spatial complementarity is a considerable determinant of the potential of sol gel selectivity. Sol gels have been employed for the preparation of catalytic materials [20]. Here it was shown that the amorphous microporous oxide retained structural memory for the kinetic diameter of the alcohol used. This is indicative of the shape of the molecule being a determinant or at least a major component of selectivity. This phenomenon has been further characterised [21]. In this study the imprinting of amorphous bulk silicas with single aromatic rings containing up to three 3-aminopropyltriethoxysilane side groups was performed. The triethoxysilane portion of the molecules side groups was incorporated into the silica framework during synthesis. The aromatic portion is cleaved thus creating a cavity in which the aminopropyl groups are spatially orientated and covalently attached to the pore surface. Leung et al. [13] have employed a tailor made organosilane – 3-[*N*,*N*-bis(9-anthrylmethyl)aminopropyltriethoxysilane as a functional monomer forming an acid base ion pair with the template 2,4-dichlorophenoxyacetic acid. The resultant sol gel material displayed good selectivity for 2,4-dichlorophenoxyacetic acid over acetic acid and benzoic acid. The authors here have concentrated on selectivity achieved by the functionalities within the pores of the sol gel rather than the shape of the cavity or hydrophobic interaction. A three monomer approach to the sol gel imprinting of lisinopril dehydrate [22] and a similar approach for 2-Aminopyridine was used [23]. It is likely that both chemical functionality and spatial complementarity of the binding cavities play significant roles in the potential selectivity of an imprinted sol gel.

This paper describes the study of the individual and combined factors which are responsible for selectivity in sol gels. The template chosen was ibuprofen, a member of the class of non-steroidal anti-inflammatory drugs (NSAIDS). Ibuprofen is indicated for rheumatoid arthritis and conditions involving inflammation. It is an inhibitor of cyclooxygenase 1, which is endogenously expressed in all human cells. In order to gain an enhanced understanding of factors affecting selectivity, three sol gels each of differing complexity and functionality have been prepared and their ability to selectively discriminate between ibuprofen and its structural and functional analogues, naproxen and ketoprofen have been studied. In the choice of functionalised siloxanes (analogous to functional monomers in MIPs) important functionalities such as hydrogen bond forming ability, electrostatic interactions and potential π–π stacking interactions have been considered. Furthermore, the two and three monomer systems offer an increased complexity in terms of the molecular size and shape of the resultant nanocavities in the sol gel. It is proposed that the major determinant of selectivity is the spatial complementarity of the cavity, however, there is a significant contribution from functional interactions within the cavity.

2. Experimental

2.1. Materials

Tetraethoxysilane (TEOS), 3-Aminopropyltriethoxysilane (APTES) and phenyltrimethoxysilane (PTMOS) were purchased from Sigma–Aldrich, Dublin and used as received. Ibuprofen ($C_{13}H_{18}O_2$, MW 206.28 g/mol), naproxen ($C_{14}H_{13}O_3$, MW 230.26 g/mol) and ketoprofen ($C_{16}H_{14}O_3$, MW 254.28 g/mol) were also purchased from Sigma–Aldrich, Dublin. All organic solvents were of HPLC grade and were purchased from Labscan, Dublin, Ireland.

2.2. Preparation of sol gels

All of the sol gels generated contained TEOS and one or both of APTES/PTMOS. The preparation consisted of two stages: firstly the hydrolysis without ibuprofen (template) and secondly condensation in the presence of ibuprofen. A 12 ml aliquot of 2-ethoxyethanol was mixed with 12 ml of TEOS. A 400 μl quantity of PTMOS and 600 μl of APTES were then added. Following this 400 μl of concentracetd HCl followed by 4 ml of water were then added and the mixture left stirring for 2 h at RT. After 2 h, 206.28 mg of ibuprofen in 20 ml water (min vol. ethanol) was added to 16 ml of the imprinting mixture and stirred for 10 min. A non-imprinted sol gel was prepared under the same conditions but with the omission of ibuprofen. The components of each of the sol gels are shown in Table 1. Condensation was allowed to progress at 80 °C for 16 h and then for 1 week at room temperature. Following this the sol gel was crushed with a mortar and pestle and sieved. Particles of 75 and 25 μm diameter were collected by sieving. The crushed sol gels were then washed to remove the template by continuous stirring in a solution of hot methanol containing 10% acetic acid. Washing was repeated until no trace of ibuprofen could be detected by HPLC.

2.3. Computational studies

The DS Viewer pro suite from Accerlys was used to calculate the molecular volumes of ibuprofen, ketoprofen and naproxen.

2.4. Physical characterisation of sol gels

The infrared absorption spectra of the three of the sol gels were obtained on a Perkin Elmer GX FTIR system. Particle size measurements were performed on a Malvern mastersizer particle size instrument by light scattering technique. A 25 mg quantity of the sol gel was exposed to 5 ml of a range of solvents for 24 h in order to examine changes in the average particle sizes of the particles.

2.5. Nitrogen sorption measurements

Pore size and surface areas of the washed sol gels were obtained by Brunauer-Emmett-Teller analysis. The analysis was performed on an ASAP 2010 from RMIT Applied Chemistry (Micromeritics). A 250 mg quantity of sol gel was used for the analysis. The sample

Table 1
The components of each of the sol gels.

Sol gel 1	Sol gel 2	Sol gel 3
2-EtOH (12 ml)	2-EtOH (12 ml)	2-EtOH (12 ml)
TEOS (12 ml)	TEOS (12 ml)	TEOS (12 ml)
APTES (600 μl)		APTES (600 μl)
	PTMOS (400 μl)	PTMOS (400 μl)
HCl (400 μl)	HCl (400 μl)	HCl (400 μl)
H_2O (24 ml)	H_2O (24 ml)	H_2O (24 ml)

was degassed for 12 h at 70 °C before analysis. Relevant information obtained from this method included surface area, total pore volume and pore diameters.

2.6. Rebinding analysis

For assessing the effect of different solvents on the % uptake of ibuprofen by the sol gels a 1 ml solution of ibuprofen at 1 μg/ml was added to 50 mg of the sol gels (and controls) in a 1 ml microcentrifuge tube in the solvents as shown in Fig. 3. The solutions were shaken for 4 h at room temperature and then centrifuged. The presence of ibuprofen in the supernatant was assayed by HPLC and the amount bound was calculated as:

Total ibuprofen − free (supernatant) = bound ibuprofen

2.7. Solid phase extraction studies

Empty solid phase extraction cartridges were washed with methanol before use. The cartridges were then dried and 200 mg of the dry sol gel (or corresponding control) was placed in between two frits. The cartridge was washed with 10% acetic acid in methanol and then with methanol 4 times until no trace of ibuprofen could be detected by HPLC. All SPE experiments were performed on a VacMaster SPE processing station manifold. Before analyte loading, the polymer was conditioned with 1 ml methanol, 1 ml acetonitrile and 1 ml water and then conditioned to the appropriate pH (pH 4–8) with water adjusted with dilute HCl. In the loading step, 1 μg/ml Ibuprofen, naproxen and ketoprofen was loaded in water at pH 4–8. For the washing step, 1 ml of 1% triethylamine (TEA) or 1% pyridine in acetonitrile or a 50:50 ratio of toluene: acetonitrile was used. Specifically bound material was eluted with 2 ml of methanol. The experiments were repeated in triplicate. After each experiment the cartridge was regenerated by washing with 3 ml of water and 3 ml of methanol.

2.8. High performance liquid chromatography (HPLC) instruments

HPLC was performed on a Hewlett Packard 1050 (HP 1050) LC system (pump, injector, detector) employing Chemstation software. The variable wavelength detector was operated at 220 nm for Ibuprofen determinations. For fluorescence applications the excitation wavelength was set at 290 nm and the emission at 350 nm. A 10 μl injection volume was used. Separations were performed on a 25 cm × 4.6 mm, 5 μm Alltech Bravda BDS C18 column.

2.9. HPLC measurements

For the Ibuprofen/Naproxen/Ketoprofen selectivity studies the mobile phase used was a 52:28:20 ratio of water: acetonitrile: methanol. The mobile phase was adjusted to pH 3.2 with phosphoric acid. For urine analysis, the mobile phase used was a 50:50 ratio of 50 mM phosphoric acid: acetonitrile.

2.10. Real sample analysis

A volunteer was given a single dose of ibuprofen (200 mg) contained in a Nurofen™ tablet. Urine samples were collected at a 6 h interval. A 1 ml aliquot of each urine sample was passed through the sol gel SPE cartridge and washed and eluted as described in Section 2.7. Free ibuprofen was quantified with reference to a standard curve. To examine conjugated ibuprofen and ibuprofen metabolites, the urine samples were hydrolysed according to the methods of [24] and [25].

3. Results and discussion

3.1. Physical and morphological characterisation of the sol gels

The three sol gels prepared were analysed by IR spectroscopy post washing. Fig. 1 shows the most distinctive infrared absorption bands related to the generation of sol gels. In the 3400–3200 cm^{-1} region, the stretching due to residual water and Si–OH stretching is observed. The band observed in all three samples at ~1050 cm^{-1} is indicative of Si–O [26] stretching while that at 930 cm^{-1} and 780 cm^{-1} can be attributed to methyl C–H stretching. Sol gels 1 and 3 both contain a small band at ~1500 cm^{-1} and this is absent in sol gel 2. This can be attributed to the incorporation of the amine group from the APTES silane (functional monomer) into the former sol gels and not the latter. The chemical nature of the polymers is unaltered by the presence of the template as there were no observable differences between each sol gel and its individual control non-imprinted sol gel (data not shown). Furthermore as has been described [15] the spectral features are consistent with organic modified silicas.

In contrast to MIPs, sol gels do not exhibit significant swelling [18]. In order to examine the potential degree of swelling further, a 50 mg amount of sol gel 3 was placed in equal volumes of a range

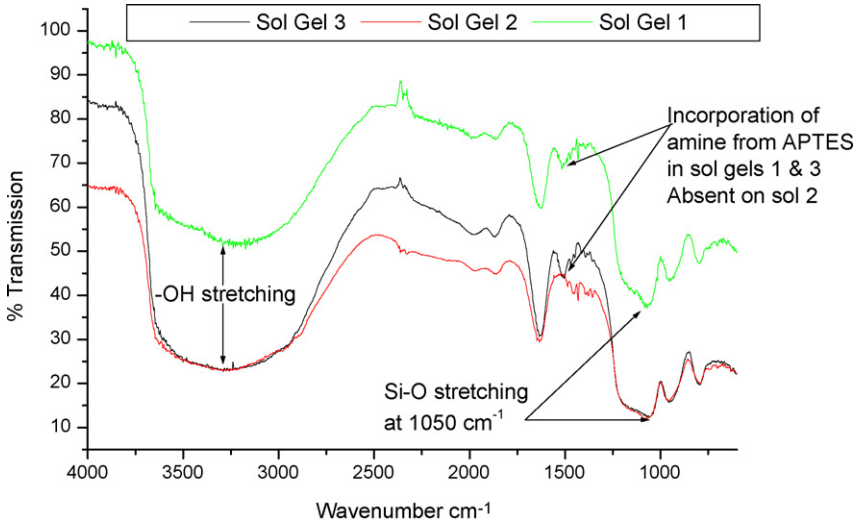


Fig. 1. FTIR absorption spectra for the three sol gels. Notable bands include the –OH at ~3250 cm^{-1} and Si–O stretching at 1050 cm^{-1}.

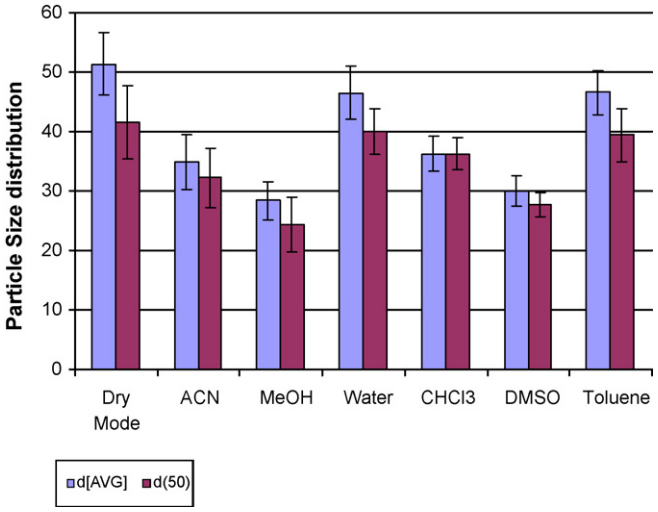


Fig. 2. The percentage shrinkage associated with particle size distribution measurements for sol gel 3. The sol gel was exposed to the solvents shown and the shrinking (relative to dry mode) is illustrated. Errors are based on the standard deviation of three experiments. The value *d*(50) is the value at which 50% of the particles in the solution lie while the value *d*[AVG] is the average particle size. Both can be interpreted as the mean of the particle sizes in a sample.

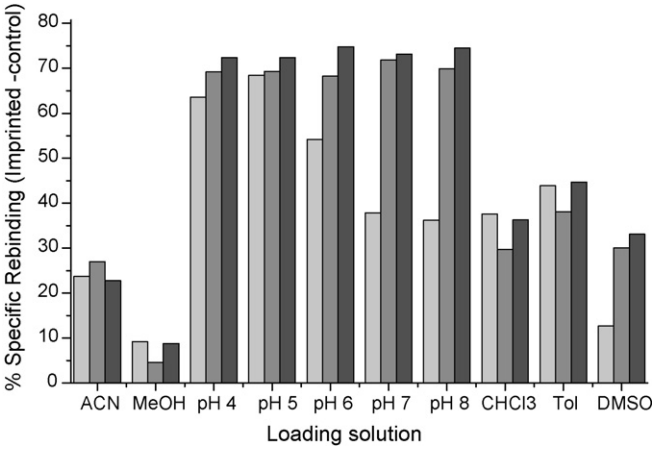
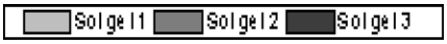


Fig. 3. Shows the specific rebinding of ibuprofen (uptake in imprinted sol gels minus that in non-imprinted) for the three sol gels under a range of organic and aqueous conditions. *Key*:



of organic and aqueous solvents and the average particle size of sol gel 3 was measured by particle size light scattering technology. The lack of swelling is an important characteristic in sol gels and will act to preserve the integrity of the binding cavity. However, sol gels have been reported to shrink [27] with an associated loss of sorption capacity. Fig. 2 shows the % shrinkage observed when sol gel 3 was dispersed in the relevant solvent for 4 h (stirring) followed by measurement of particle size. It was found that water caused the least reduction in average particle size and nonpolar solvents lead to only minimal shrinkage. Both polar protic (methanol) and polar aprotic (ACN and DMSO) cause greater observed shrinkage of the sol gel. It is somewhat surprising that the more polar solvents are observed to lead to greater shrinkage of the sol gel than the nonpolar solvents. Collinson [28] has reported that during drying, alcohol evaporates from the pores causing the sol gel to shrink. In this instance it is likely that the polar solvents diffuse into the pores and cavities of the sol gel replacing residual water and evaporate during the measurements leading to continued shrinking of the material. The nonpolar solvents may not diffuse into the pores as efficiently and hence their loss through evaporation has less of a shrinking effect on the pores. It is important to note that while sol gel materials do not swell to the same degree as conventional imprinted materials, their extended use at pH > 8 is likely to lead to their destruction.

3.2. Initial rebinding studies

Fig. 3 shows the relative specific uptake of ibuprofen i.e. % rebinding in the sol gels minus that in the control sol gels for the three sol gels prepared. It was found that all three of the sol gels demonstrate enhanced rebinding in aqueous conditions. Furthermore, there is a cline of decreased rebinding given that the sol gels perform best in aqueous conditions followed by nonpolar (chloroform, toluene) then polar aprotic (acetonitrile, DMF) and then polar protic (methanol and ethanol). A similar trend has been noted by other authors [12]. In this study, it was pointed out that the increased rebinding of propanolol to the sol gel could result from the preferred solubility of the molecule in organic media relative to aqueous and this is in part justified by the partition coefficient of propanolol in the octanol/water system. Nonpolar solvents such as chloroform would not be expected to interfere with hydrogen bonding whereas more polar solvents will form strong hydrogen bonds with the template thus precluding the formation of specific interactions with the functionalities in the pores [23].

3.3. Effect of pH on rebinding

Fig. 3 also describes the effect of variation of pH on rebinding. Firstly, in regards to sol gel 1 (prepared with the APTES monomer), at pH 4 and 5 the rebinding is significantly greater than at pH 6–8 (>60% specific rebinding as opposed to 40–50%). Since the significant method of complex formation of ibuprofen with APTES is hydrogen bonding, a negatively charged molecule will be unable to participate in hydrogen bonding and hence this type of interaction is inhibited at higher pH where the molecule will be deprotonated. Some rebinding will still occur to the sol gel at higher pHs because of the presence of van der Waals, electrostatic forces, shape complementarity along with interactions of ibuprofen with the silanols of the sol gel. For these reasons an abrogation of rebinding will not be observed. This is further exemplified by the rebinding in aqueous conditions to sol gels 2 as shown in Fig. 4b (and sol gel 3) as shown in Fig. 5. Here the monomer used was PTMOS which will form π–π stacking or hydrophobic interactions with ibuprofen and the nature of these interactions will not be significantly altered by changes in pH in the region 4–8. As such, a greater level of rebinding of ibuprofen to sol gels 2 and 3 is observed at all pHs examined.

Sol gel 3 contains both APTES and PTMOS as functionalised silane monomers. As part of the three monomer system, the significance of the hydrogen bonding interaction with APTES is reduced relative to other determinants of selectivity such as shape complementarity, π–π stacking and hydrophobic interactions and hence these interactions can to a large extent compensate for the loss of the hydrogen bonding. The rebinding studies have shown that there are number of factors which combine to reach optimum binding conditions for all of the sol gels prepared which can be accounted for individually.

3.4. Selectivity studies using solid phase extraction

In order to analyse the individual and collective factors responsible for rebinding ibuprofen and for selectivity for ibuprofen over the structural analogues naproxen and ketoprofen, solid phase extraction (SPE) was performed utilising a range of loading and washing (desorption) conditions. Initially the desorption conditions were analysed to examine which washing conditions led to

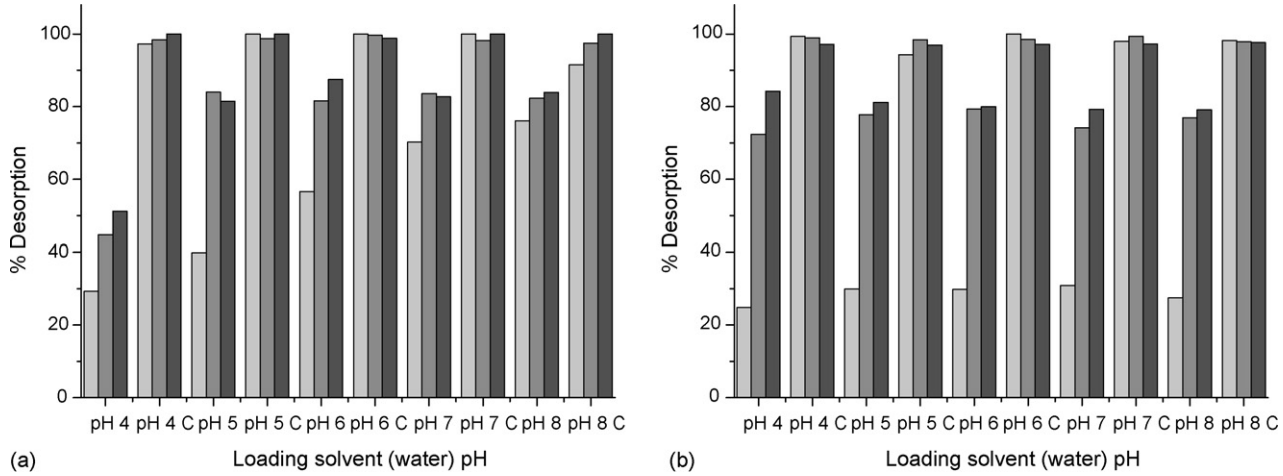


Fig. 4. Selectivity study on sol gel 1 (a) and sol gel 2 (b). The % desorption from each of the sol gels was studied on washing with 1% pyridine under loading conditions ranging from pH 4–8. *Key*: Ibuprofen Naproxen Ketoprofen.

optimal retention of the analyte of interest (ibuprofen) on the sol gel columns while minimising the cross reactivity.

Non-imprinted controls were also used to determine the specificity of the molecularly imprinted sol gels. For all of the sol gels and controls equal concentrations (1 μg/ml) of ibuprofen, naproxen and ketoprofen were loaded in under aqueous conditions at pH 4–8. A 1% solution of pyridine in water was used as the washing solvent in order to act as a competing amine for non-specifically bound material. This procedure was applied to all of the sol gels. Fig. 4a (sol gel 1), 4b (sol gel 2) and 5 (sol gel 3) shows the result of this approach. At a loading and washing pH of 5, the quantity of ibuprofen desorbed from the sol gel column is approximately 2.5 times less than both naproxen and ketoprofen. At pH 4 the effect is somewhat reduced but selectivity for ibuprofen over the analogues is still noticeable. Interestingly, as the pH of the loading and washing solutions increase, the selectivity for ibuprofen over the analogues is significantly reduced.

3.5. Contribution of hydrophobic interactions to selectivity

Sol gel 2 contained PTMOS as the functional monomer with the concomitant ability to form π–π stacking (or hydrophobic) interac-

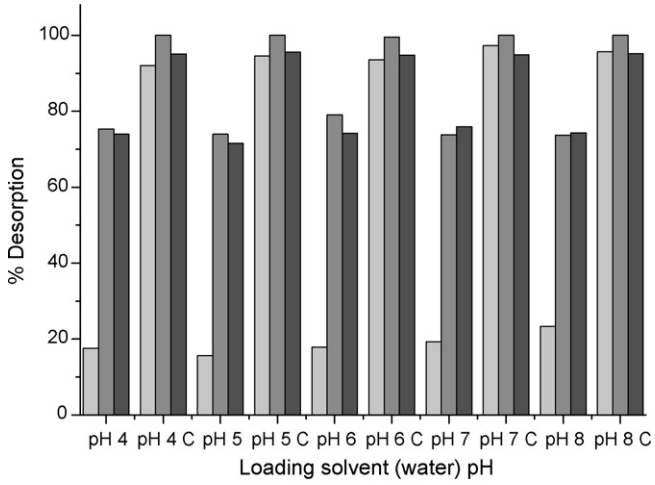


Fig. 5. Selectivity study on sol gel 3. The % desorption was studied on washing with 1% pyridine. *Key*: Ibuprofen Naproxen Ketoprofen.

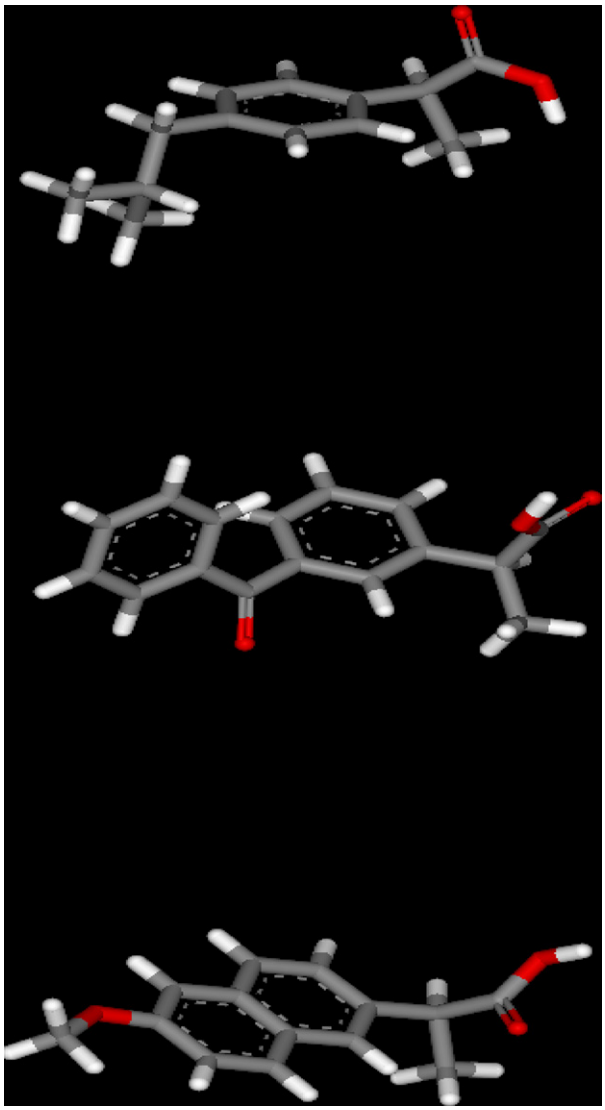


Fig. 6. The three-dimensional structures of the molecules used in the study ibuprofen (above) ketoprofen (middle) and naproxen (below).

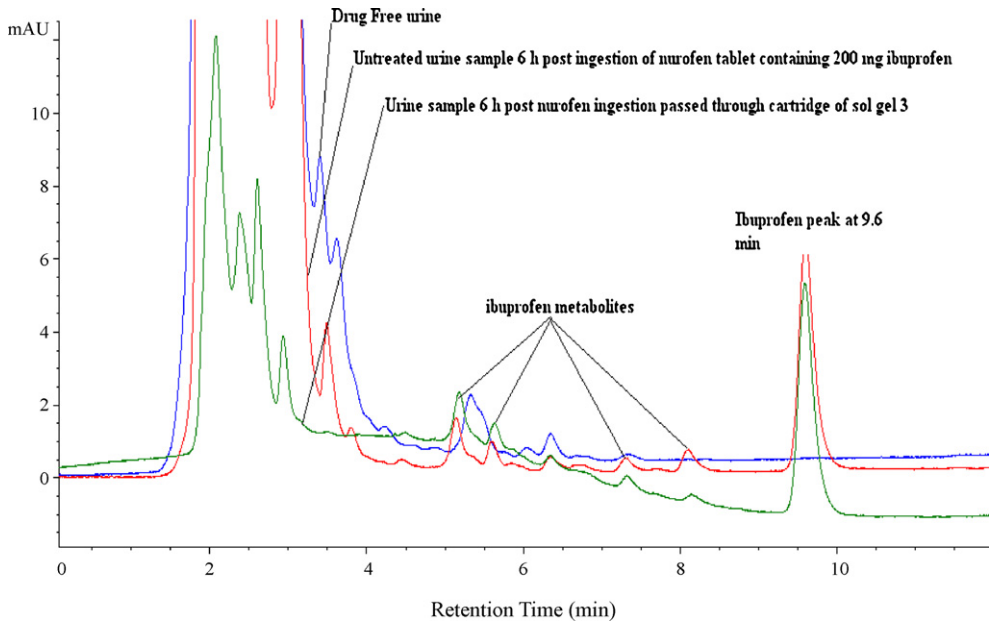


Fig. 7. HPLC chromatograms obtained following solid phase extraction of urine sample 6 h post ingestion of 200 mg ibuprofen using 200 mg of sol gel 3. Ibuprofen eluted at 9.6 min with the more polar unidentified species possibly the metabolites between 5.0 and 8.5 min.

tions with ibuprofen. However both naproxen and ketoprofen also contain aromatic rings, which could increase the probability of cross reactivity. Pyridine was again chosen as a component of the washing solution due to that fact that it would disrupt non-specific interactions by forming interactions with weakly bound material i.e. naproxen and ketoprofen and also to act as a competing amine for material which may be non-specifically bound. When 1% pyridine in acetonitrile was used as the washing solution, a large portion of the ibuprofen was removed from all of the sol gels. It was noticeable however that a larger proportion of the analogues were removed also. This is attributed to two reasons. Firstly as already mentioned, acetonitrile is a powerful eluting solvent but secondly, the nature of π–π stacking is that it is a weak interaction (significantly weaker than hydrogen bonding). As a result of this a 1% solution of pyridine in water was applied as washing solution. For sol gel 2, Fig. 4b shows that when using this washing solution maximal selectivity for the sol gel towards ibuprofen was achieved. The relative strength of the mechanisms of interaction will differ considerably between the molecules. The three-dimensional structure of the three analogues is shown in Fig. 6. Ibuprofen contains a single aromatic ring with no electron withdrawing substituents directly on the ring. The shape of the molecule also affords steric manoeuvrability to a potential aromatic ring coming into close contact and allowing the formation of the π–π electron delocalisation necessary. Regarding the three-dimensional structure of naproxen the naphthyl group will be significantly more stable than the single ring of ibuprofen. The result of this is that the electronic distribution will be more stable and hence will not participate as readily in a π–π stacking arrangement. Regarding ketoprofen, the molecule possesses a carbonyl group between the two aromatic rings. The electron withdrawing nature of this substituent will adversely affect the ability of the ketoprofen aromatic rings to form π–π stacking arrangements with PTMOS. Hence, the strength of π–π stacking interactions between the PTMOS ring and each of the analogues will differ.

3.6. Shape complementarity

There is considerable difference in both size and shape between the three molecules used in this study. The Acceryls program calculated the molecular volume of ibuprofen to be 149.8 Å^3 with that of naproxen at 151.0 Å^3. Ketoprofen has a molecular volume of 174.8 A^3. In the field of molecularly imprinted polymers, the work of Spivak and co-workers [29] has shown the importance of shape selectivity in non-covalently imprinted polymers. For sol gels, it has been demonstrated [21] that imprinted silica could act as a shape selective base catalysts. To study this further a third sol gel (sol gel 3) was prepared using both APTES and PTMOS. It was expected that the resultant nanocavity would be even more size and shape selective for ibuprofen. As is shown in Fig. 5a, the selectivity for ibuprofen over naproxen and ketoprofen showed a marked increase over using APTES or PTMOS alone as the functional monomer. The binding of catecholamines on imprinted on silica–alumina gel shows a size selective effect [30]. Compared to norepinephrine and epinephrine, the smallest size of dopamine imprinted cavities only allowed rebinding to dopamine itself. Shape complementarity assumes an increased significance when a competing molecule to the analyte of interested is smaller in size or bulk (or at least of similar size). If the competing molecule was larger then it would be excluded from the binding cavity due to steric hindrance as is evident from the high level of desorption of ketoprofen.

Essentially, a molecule of similar size but difference shape i.e. ibuprofen will be allowed to manoeuvre within the naproxen sol gel binding cavity but with sub-maximal binding. The phenomenon is described as a “non-optimal spatial fit” with reference to MIPs. In effect, if a molecule lacks optimal shape complementarity to the spatial organisation of the binding cavity, the number of potential contact interaction points will be reduced. Since the optimal spatial fit is not achieved, rebinding will have to overcome an extra thermodynamic barrier hence a lower level of rebinding will be observed. The molecule exhibiting the optimal spatial fit, in this will demonstrate a better fit to the shape of the binding cavity and thus the sol gel (sol gel 3) shows enhanced selectivity towards ibuprofen. Despite the similar molecular volumes of ibuprofen and naproxen, the shapes of the molecules are significantly different. Hence, a molecularly imprinted sol gel prepared against ibuprofen demonstrates selective rebinding on the order of ibuprofen $\gg$ naproxen > ketoprofen.

3.7. Application to real samples.

To demonstrate the applicability of the molecularly imprinted ibuprofen sol gel (sol gel 3) it was employed in a solid phase extraction procedure. A urine sample was taken from a volunteer at 6 h post ingestion of a single dose of 200 mg of ibuprofen contained in a Nurofen™ tablet. Ibuprofen undergoes extensive conjugation in the human body with the kidney being the main source of excretion. Only 1% of an ingested dose of ibuprofen is excreted as the free i.e. unconjugated molecule. Two major metabolites of ibuprofen are produced—carboxyibuprofen and hydroxyibuprofen for each of which exists stereoisomers. A 1 ml volume of urine at the above time point was loaded independently onto the sol gel column without pH adjustment. Although, selective clean up of the urine sample was achieved, a small recovery of ibuprofen along with several other peaks—most likely the analogues carboxyibuprofen and hydroxyibuprofen was obtained. This was attributed to the significant glucuronidation of ibuprofen. Given the large size of this molecule it will not be compatible with the spatial complementarity afforded by the sol gel cavity. In order to free the ibuprofen, the urine sample was subjected to a hydrolysis reaction as described [24,25]. This led to greatly increased peak areas for ibuprofen and also the unidentified peaks for the same sample which further indicates the probability of these peaks being those of the ibuprofen metabolites. The above procedure was repeated using the same loading and washing conditions as before. As can be seen from Fig. 7, quantitative clean up of the urine sample was achieved and the amount of unmetabolised ibuprofen in the sample at 6 h post ingestion was quantified at 7.07 μg/ml. Given the close structural similarity between ibuprofen and the two major metabolites, it is likely that the sol gel did not discriminate significantly between the metabolites. At a loading pH of 6–7, it was noted that the sol gel had greater retention of unidentified compounds over ibuprofen. This is likely due to the fact that both of the metabolites contain an extra hydroxyl group and will still be capable of undergoing hydrogen bonding with the APTES functionality while at pH 6–7 ibuprofen will be fully deprotonated. This is the most likely working hypothesis as the spatial differences between ibuprofen, carboxyibuprofen and hydroxyibuprofen are minimal.

4. Conclusion

The objective of this study was to enhance the understanding of the nature of selectivity in molecularly imprinted sol gels. This was achieved in two ways. Firstly, the physical characterisation of sol gels generated using different functional silanes and secondly by probing the selective rebinding abilities of each of the sol gels by varying experimental rebinding conditions and determining the optimum solid phase extraction procedure to extract ibuprofen from a mixture of ibuprofen, ketoprofen and naproxen. It was proposed that there are two determinants of selectivity in these sol gels. Firstly the chemical functionality imparted to the sol gel by the functional silane e.g. APTES, PTMOS and secondly the specific geometric binding cavity which through spatial complementarity serves as a highly selective shape based exclusion cavity which allows entry of the analyte of interest (template) whilst refusing access to even analogues of the template depending on the level of spatial similarity of the analogue(s) with the template.

Furthermore, the potential applications of such highly selective sol gels have been demonstrated by solid phase extraction clean up and preconcentration of ibuprofen (and metabolites) from urine. The applications of this type of material are many and can be performed easily and economically. It is demonstrated that by understanding the nature of sol gel chemistry, specific and selective materials can be produced that can easily be used as solid phase sorbents or incorporated into analytical devices.

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