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Analyte adsorption in liquid chromatography valve injectors for samples in non-eluting solvents

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Abstract

The cause of precision problems arising when using non-eluting solvents for sample solutions in order to obtain a peak compression effect in low-dispersion liquid chromatography was investigated. Quantitative evidence was obtained to support the proposition that the problems were caused by adsorption of the analyte in the injection system. This phenomenon was quite marked when aqueous buffer was used to inject into organic–aqueous mobile phases and could still be significant when there was only a few percent difference in the organic content of the mobile phase and the less eluting injection solvent. While there was evidence for adsorption onto the inner walls of the stainless-steel tubing used for sample loops, it was clear that, for this case of liquid chromatography of indomethacin, adsorption onto Vespel rotor seals was the principal contributing factor to the effect.

Keywords: Adsorption; Injectors; Valve injectors; Indomethacin

1. Introduction

Over the past decade or more there has been increased interest in miniaturised LC columns with either reduced length or cross-sectional area. In such columns there is low dispersion of the sample band between the point of injection and the exit of the column [1–5]. In many instances, low-dispersion LC must be used in conjunction with the technique of peak compression whereby the use of a non-eluting solvent gives rise to focusing of the sample band at the top of the column.

While there are several reports of the successful use of peak-compression phenomena in low-dispersion LC [6–8] there remains a need for a systematic study of this effect. It was found during the development of an assay intended for use in such a study [9] that the precision of repeat injections of the same solution was somewhat less than expected. Similar problems have been encountered by other workers when using sample solvents which were less strongly eluting than the LC mobile phase [10–14]. Kirschbaum and Perlman [10] noticed changes in peak areas of aztreanan depending on whether the sample was injected in water or mobile phase. Inman et al. [11] found that the detector response to vancomycin and vinblastine was dependent on the solvent strength of the sample solution. Macleod et al. [12] found that during chromatography of an anxiolytic agent, the concentration of acetonitrile in

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the sample solution affected the observed peak height and suspected that this was related to sample adsorption on the valve rotor seal. On the other hand, Fernandez Otero et al. [13] interpreted their data as indicating that, while sample adsorption was compound-dependent, for the range of compounds they tested adsorption took place in the loop rather than on the rotor seal. The overall impression gained from these literature reports was that there was a general feeling that analyte adsorption within the injection system was the cause of the problems being observed but the exact location of the adsorption was not always clear [14].

In our own work other anomalies in the use of peak compression were found [15] which did not seem to be directly related to the poor precision, but for which, similarly, adsorption seemed the most likely cause of the problem. The objective of the work reported here was therefore to seek further evidence to support the proposition that analyte adsorption in the injection system was the cause of the problems observed by ourselves and other workers. It was also intended to find out more about the extent of this phenomenon and to do more to pinpoint its exact source.

2. Experimental

2.1. Instrumentation

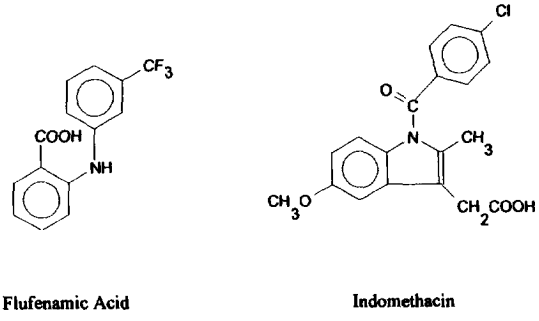
The chromatographic system consisted of a Shimadzu LC-10AD pump, SPD-6AV UV–Vis detector fitted with an 8-μl flow cell and C-R5A integrator (all from Dyson Instruments, Houghton-le-Spring, UK). A Rheodyne 7125 injection valve fitted with a Vespel rotor seal and stainless steel 5-μl and 10-μl external loops were used. The dimensions of the stainless-steel connecting tubing was 90×0.18 mm pre-column and 90×0.18 mm post-column (Anachem, Luton, UK). A Spherisorb ODS (5 μm), 12 cm×2 mm I.D. stainless-steel column (Capital HPLC, Broxburn, UK) was used with a Spherisorb (10 μm), 10 cm×4.6 mm I.D. silica pre-column in line before the injector. The column temperature was maintained at 30°C with a water jacket and Tecam TE-7 Tempette pump/heater (B.D.H., Poole, UK). The detector wavelength was 254 nm, the integrator chart speed was 5 mm min^{-1} and 60 mm min^{-1} during determinations of column efficiency.

2.2. Materials and methods

Vespel (carbon impregnated polyimide) and Tefzel [ethyltetrafluoroethene (ETFE)] Rheodyne injector valve rotor seals (Anachem) were used. Water was glass-distilled and de-ionised (Milli-Q purification system, Millipore, Watford, UK). Methanol was purchased from Rhone-Poulenc (Manchester, UK) and disodium hydrogen orthophosphate, AR grade and orthophosphoric acid (85%), GPR from B.D.H. Indomethacin, ibuprofen and flufenamic acid were obtained from Sigma (Poole, UK). For structures of the compounds see Fig. 1.

2.3. Procedures

The mobile phase, methanol–0.02 *M* phosphate buffer (pH 7.0) (58:42, v/v) was filtered and degassed under vacuum before use. The flow-rate used throughout was 0.378 ml min^{-1}. Indomethacin solutions, 1 μg ml^{-1} and 10 μg ml^{-1}, were prepared in 0.02 *M* phosphate buffer (pH 7.0), mobile phase and a range of other methanol–aqueous buffer solutions. These solutions were injected onto the chromatographic system described above and peak-height and area data were obtained. The volume of solution used to overfill the sample loops was varied and in some experiments the solution was washed out of the loop before switching to the 'inject' position.



Flufenamic Acid **Indomethacin**

Fig. 1. Structures of flufenamic acid and indomethacin.

2.4. Relative comparison of the degree of adsorption between stainless steel, and Vespel and Tefzel rotor seals

Into three identical screw-capped vials each containing 5 ml of indomethacin in phosphate buffer, 10 μg ml^{-1}, was placed: (a) a Vespel rotor seal, (b) a Tefzel rotor seal, (c) the stainless-steel ring removed from another Vespel rotor seal. These parts were used as supplied without previously having been put to use in a LC injection valve. A fourth vial (d) contained only 5 ml of indomethacin solution as a control. After standing for 3 h, with occasional agitation, the solutions in each vial were sampled and 10-μl injections made onto the LC column.

The seals and stainless-steel ring were then removed from the indomethacin solutions and washed rigorously with water and placed into three screw-capped beakers each containing 1 ml of mobile phase to desorb any indomethacin still remaining. Samples of 10 μl were then removed and injected during the following hour. (The experiment was not repeated using seals without a stainless-steel ring since it was found to be very difficult to remove the rings without badly scratching the surfaces of the seals. This would have altered the capacities of the seal compounds for sample adsorption in ways which would have been difficult to reproduce.)

Peak heights were taken and expressed in terms of μg of indomethacin on column by comparison with the peak heights obtained for the control sample. The results were then compared with the calculated mass of indomethacin which was observed to have been adsorbed in the first phase of this experiment.

3. Results and discussion

The possibility of analyte adsorption taking place in the injection system when carrying out peak compression for LC of indomethacin was investigated by passing increasing volumes of a solution of 1 μg ml^{-1} of indomethacin in an aqueous phosphate buffer through a Rheodyne injector fitted with a 10-μl sample loop, i.e. using the common practice of loop overfill. The results (Fig. 2) show an increase in peak height with the volume of solution passed through the injection valve as would be expected if

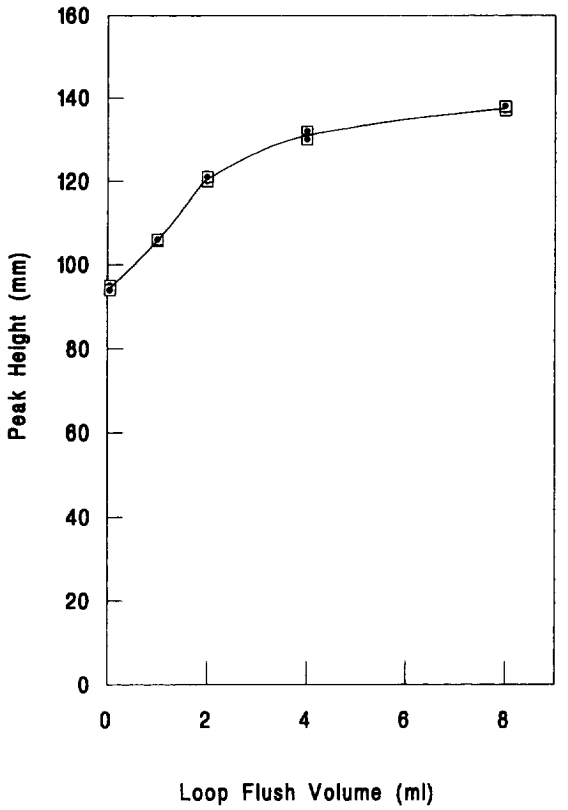


Fig. 2. Effect of increasing loop flush volume of 1 μg ml^{-1} indomethacin solution in 0.02 *M* phosphate buffer (pH 7.0) through injector and 10-μl loop. Mobile phase: methanol–0.02 *M* phosphate buffer (pH 7.0) (58:42, v/v), flow-rate 0.378 ml min^{-1}; column: Spherisorb ODS (5 μm), 12 cm×2 mm I.D.

adsorption were taking place. As expected no such effect was observed when mobile phase was used as the sample solvent. Taking the extreme case of an 8.0-ml loop flush volume the observed increase in peak height was equivalent to a column loading of 15.6 ng as opposed to the loading of 10 ng which should arise from 10 μl of a solution of 1 μg ml^{-1}. More importantly, a similar experiment using a 5-μl loop introduced a +36% bias on increasing the flush volume from 25 μl to 50 μl, which is a more common flush volume. Clearly this could be a significant problem in a quantitative method.

If analyte adsorption was taking place in the injection system the observed peaks would consist of a component arising from analyte desorbed from the valve as well as the component arising from 10 μl of sample solution. This was more clearly demonstrated

by eliminating the 10-μl sample solution component. Following loading with indomethacin solutions, the injection system was flushed with 1 ml of aqueous phosphate buffer before switching from the 'load' to the 'inject' position. Peaks for indomethacin were still observable (Fig. 3) and as before, increased with the loop flush volume of indomethacin solution. It was also observed that the increase in peak heights observed was not so large as the increases in peak heights observed in the experiment in which loop flushing with aqueous phosphate buffer was not carried out. This indicated that there was a limited amount of desorption during the aqueous buffer loop flush. This was confirmed by maintaining the same loop flush volume of indomethacin solution and varying the volume of aqueous buffer flush (Fig. 4).

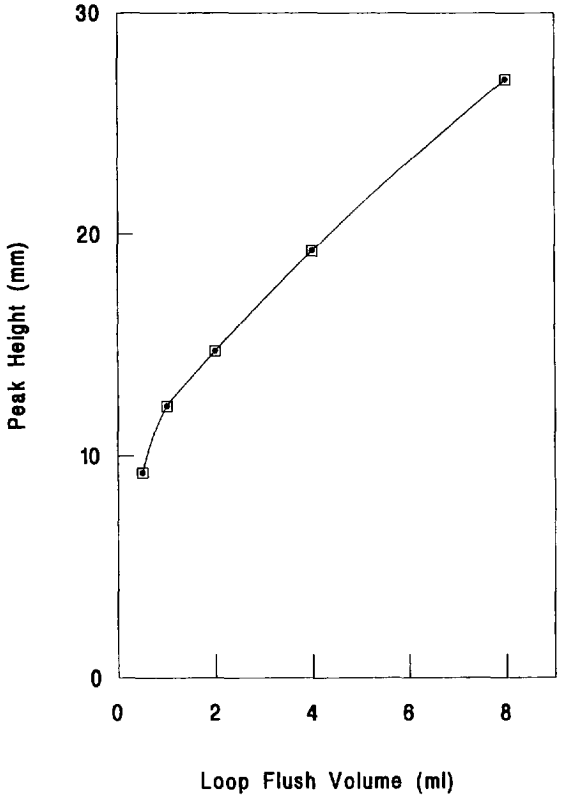


Fig. 3. Effect of increasing loop flush volume of 1 μg ml^{-1} indomethacin solution in 0.02 *M* phosphate buffer (pH 7.0) through injector and 10-μl sample loop. Valve and loop were flushed with 1 ml blank aqueous buffer after loading the sample and before each injection to remove the indomethacin solution from the loop. Chromatographic conditions as in Fig. 2.

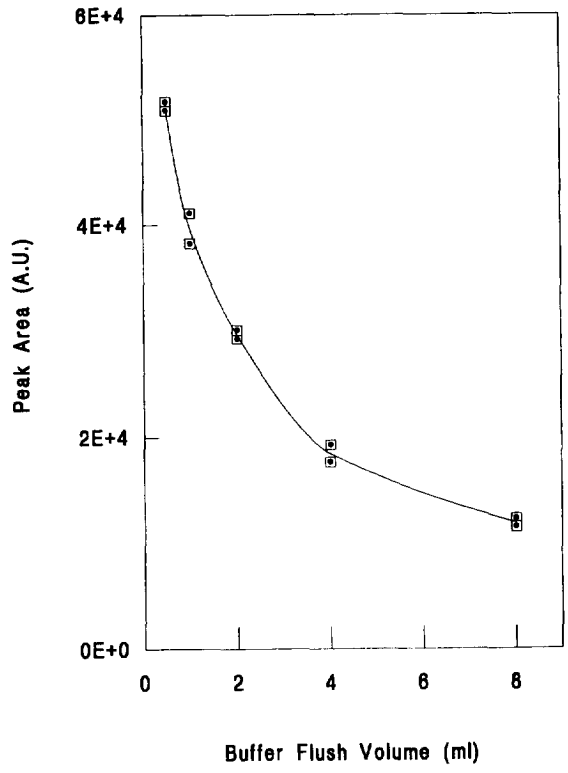


Fig. 4. Effect of increasing buffer flush volume after loading sample solution. A 1-ml indomethacin solution of 1 μg ml^{-1} in 0.02 *M* phosphate buffer (pH 7.0) was passed through injector and 10-μl sample loop. Valve and loop were then flushed with increasing volumes of blank aqueous phosphate buffer after loading the sample and before each injection to remove the indomethacin solution from the loop. Chromatographic conditions as in Fig. 2.

The observed response to indomethacin decreased as the volume of buffer flush increased. When the loop was flushed with 1 ml of mobile phase instead of 1 ml of aqueous buffer no peaks were observed. These observations further suggested that sample adsorption was taking place and that the process was reversible and dependent on the solvent strength of any solvent passed through the injector.

Another consequence of analyte adsorption in the injection system should be that, given the limited surface area therein, it should be possible to reach a loop flush volume at which saturation occurred, beyond which there was no further increase in indomethacin peak height. The flattening off of peak height at higher volumes as shown in Fig. 2 might

have been consistent with the onset of saturation. However it was felt that more conclusive evidence was required. This evidence was obtained (Fig. 5) by using a higher concentration (10 $\mu g\,ml^{-1}$) of indomethacin so that saturation could be demonstrated without having to use unattainably large volumes as might have been the case for the solution of 1 $\mu g\,ml^{-1}$. Solubility problems precluded using higher concentrations than 10 $\mu g\,ml^{-1}$. Even with the solution of 10 $\mu g\,ml^{-1}$ a flush volume in the order of 30 ml was required before saturation was reached. Although this was an extreme case in which adsorption was being encouraged because of the saturation of the sample solution, it still showed that the injection system had quite a high capacity for

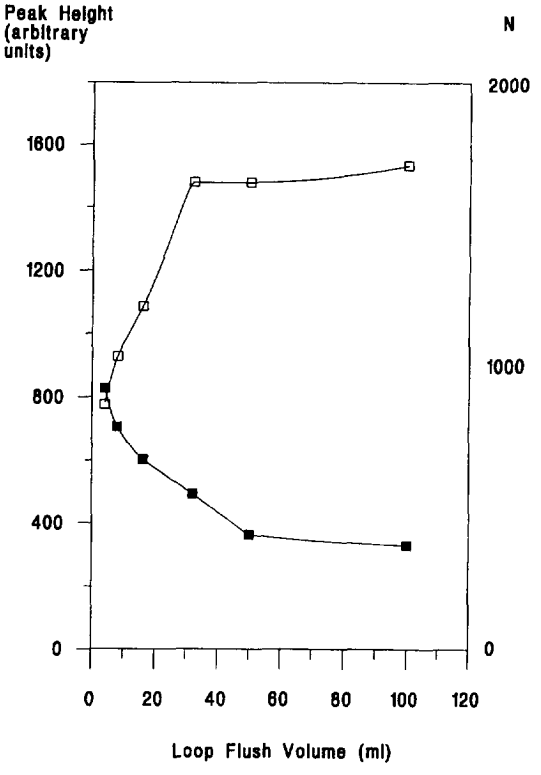


Fig. 5. Effect of increasing loop flush volume of a solution of 10 $\mu g\,ml^{-1}$ indomethacin in 0.02 *M* phosphate buffer (pH 7.0) through injector and 10-μl sample loop. Valve and loop were then flushed with 1 ml of blank aqueous phosphate buffer after loading the sample and before each injection to remove the indomethacin solution from the loop. Chromatographic conditions as in Fig. 2. (■) Effect on column efficiency (*N*) of increasing the loop flush volume; (□) effect on peak height of increasing the loop flush volume.

adsorbing indomethacin. In this case about 60 ng remained adsorbed in the system even after a 1-ml aqueous buffer wash was applied. As indicated in Fig. 4 this quantity would have been considerably larger had the injector not been flushed with aqueous buffer before injection. This value will vary from injector to injector depending on the total surface area of adsorbing materials. This in turn will vary with the state of repair of the injector or perhaps the correctness of the alignment of the internal parts. One interesting feature of this saturation experiment was the marked loss of efficiency with increasing loop flush volume (Fig. 5). This drop in efficiency was clearly not caused by factors such as mass overload on the column, since there had been no loss in efficiency when higher sample masses were injected in low-volume loops with partial loop filling. It could be explained more readily by the finite time that would be required to desorb indomethacin from its sites in the injector. Similarly, loss of efficiency through slow desorption may be one of the contributory factors in relation to the findings of others [16] that the practice of 'peak clipping' (i.e. returning the injection valve directly back to the load position the very instant sufficient time has elapsed for the contents of the loop to have been expelled) when using the peak-compression effect gave improved efficiency. The relatively slow adsorption/desorption process also manifested itself in that peak heights obtained showed some variation if deliberately wide variations were made in the speed at which the indomethacin solution was flushed through the loop. Further confirmation of adsorption/desorption taking place was obtained by more clearly demonstrating the effect of residence time (Fig. 6). The delay time before switching the valve to the inject position was varied, thereby varying the time the analyte spent in contact with the adsorbing surfaces. The results (Fig. 6) demonstrated that over two minutes were required for equilibrium to be reached between indomethacin in solution and indomethacin on adsorbing surfaces.

Using aqueous buffer solutions to bring about peak compression could be considered to be an extreme case since it might still be possible to bring about significant peak compression by using higher-strength solvents containing some methanol so long as the ratio of methanol to buffer was less than for

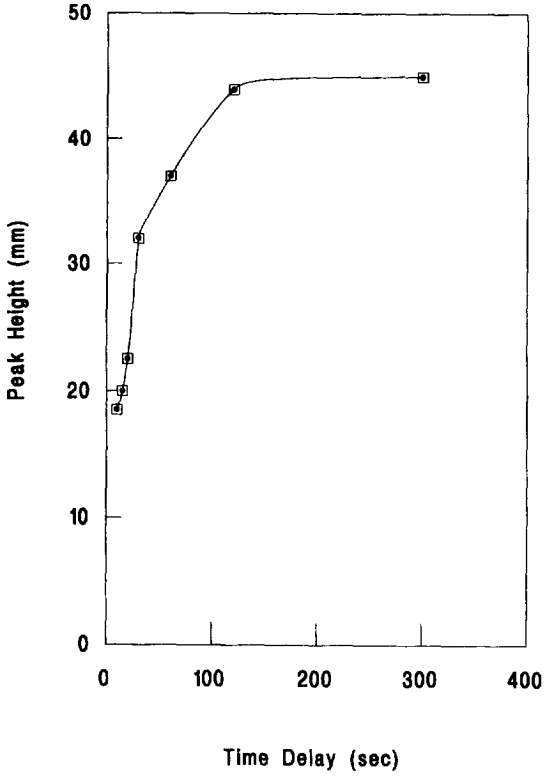


Fig. 6. Effect of increasing time delay before injection. A 1-ml indomethacin solution of 1 μg ml^{-1} in 0.02 *M* phosphate buffer (pH 7.0) was passed through injector and 10-μl sample loop. The solution was then left in the valve and loop for increasing time periods and then flushed with 1 ml of blank aqueous phosphate buffer before each injection to remove the indomethacin solution from the valve and loop. Chromatographic conditions as in Fig. 2.

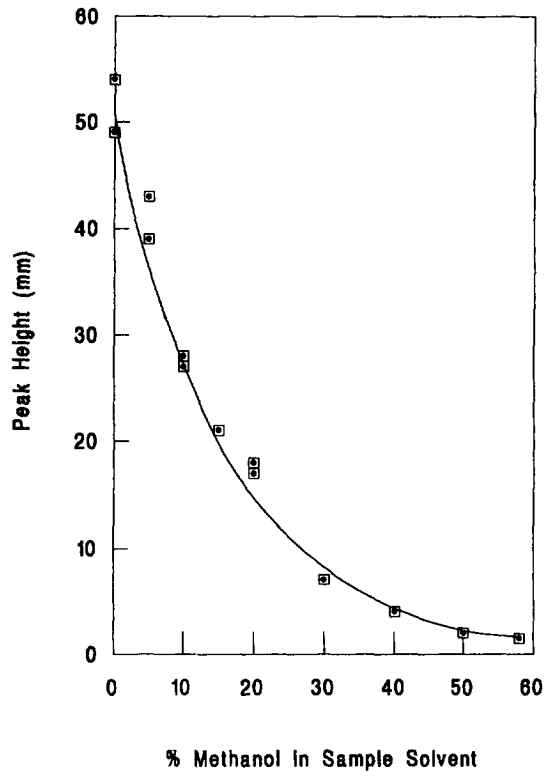


Fig. 7. Effect of increasing % methanol concentration of the sample solvent. As amount of 1 ml of indomethacin solution 1 μg ml^{-1} in 0.02 *M* phosphate buffer (pH 7.0)/methanol was passed through injector and 10-μl sample loop. Valve and loop were then flushed with 1 ml of blank aqueous phosphate buffer after loading the sample and before each injection to remove the indomethacin solution from the loop. Sample loop 10 μl. Chromatographic conditions as in Fig. 2.

the mobile phase. It was therefore sought to determine whether the effects which seemed to be associated with analyte adsorption in the injector were still prevalent when using higher-strength solvents.

A series of standard solutions of indomethacin, 1 μg ml^{-1}, were made using a range of sample solvent strengths from 100% buffer, to a methanol–buffer ratio of 58:42. Volumes of 1 ml of these solutions were passed through the injector and loop and injections made (Fig. 7).

These results show that the extent of the sample adsorption was reduced with increasing methanol concentration. It may also be noted that adsorption began when there was only a small percentage reduction in the methanol concentration compared with the mobile phase and this effect became increasingly important as the methanol concentration was further reduced.

At this stage it was unclear whether or not the adsorption effect was specific to indomethacin. Also, in order to devise methods to circumvent adsorption, it was necessary to find out which part(s) of the injector were responsible for it. With respect to the latter an experiment was set up wherein the analyte solution was exposed to the stainless-steel loop only. With respect to the former, the experiment was carried out using flufenamic acid, another non-steroidal anti-inflammatory drug (NSAID), less hydrophobic than indomethacin. A series of injections of increasing sample volume were therefore passed

through a Rheodyne 7125 injector fitted with a 10-μl sample loop. Once the loop had been flushed with aqueous buffer it was detached from the injector, fitted to a second Rheodyne 7125 injector and then flushed again with aqueous buffer prior to switching to the inject position to allow mobile phase to pass through the loop on its way to the column. It was found (Fig. 8 and Fig. 9) for both indomethacin and flufenamic acid, that peaks were still observed under these experimental conditions under which no analyte had come into contact with the internal components of the injection valve. The peak heights, however, were smaller than those obtained for the similar experiment in which the analyte had been passed through both the injector body and the loop. The implication was therefore that while analyte adsorption in the injection system took place on the inside walls of the stainless-steel loop, it took place to a much greater extent inside the injector body. This could have been caused by adsorption onto any exposed stainless-steel surfaces within the injector body. However, especially since any such stainless-steel surface would not be likely to be greater in area than the stainless-steel surface in the loop, it is likely that analyte adsorption in the injector body was onto a different material, almost certainly the polymer of which the rotor seal was made.

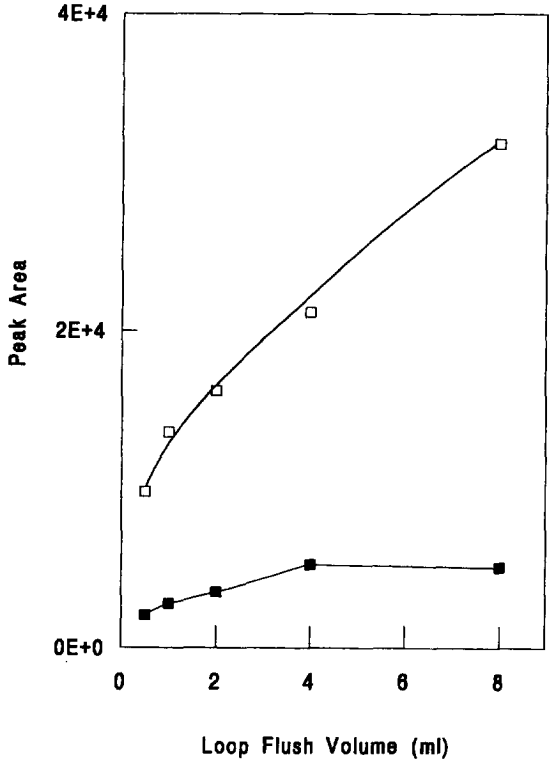


Fig. 8. Contribution to observed increased response to indomethacin by injection valve and sample loop. Increasing loop flush volumes of 1 μg ml^{-1} indomethacin solution in 0.02 *M* phosphate buffer (pH 7.0) was passed through injector and 10-μl sample loop and through the sample loop only. Valve and loop were then flushed with 1 ml blank aqueous buffer after loading the sample and before each injection to remove the indomethacin solution. Chromatographic conditions as in Fig. 2. (□) Contribution to observed response by sample loop and injector; (■) contribution to observed response by sample loop only.

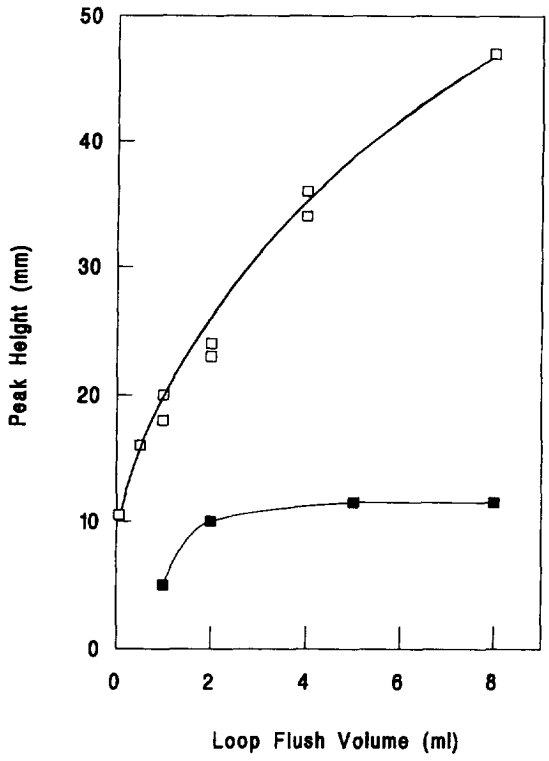


Fig. 9. Contribution to observed increased response to flufenamic acid by injection valve and sample loop. Experimental details as in Fig. 8, but flufenamic acid was substituted for indomethacin. (□) Contribution to observed response by sample loop and injector; (■) contribution to observed response by sample loop only.

This was corroborated by changing rotor seals. In changing from a Vespel rotor seal to a Tefzel rotor seal it was found that while indomethacin adsorption still took place it was reduced by around 40%. This was demonstrated even more conclusively in experi-

Table 1
Effect of exposure to isolated rotor seals on peak area of indomethacin

	Vespel	Tefzel	Steel ring	Control
Area (A.U.) $\times 10^3$	629	645	642	664
S.D.	11.3	17.4	14.9	5.26
C.V. (%)	1.8	2.7	2.3	0.8
n	8	8	8	8

Injection of 100 μl through a 10-μl sample loop of an indomethacin solution (1 μg ml^{-1} in 0.02 M phosphate buffer (pH 7.0) which had been exposed to isolated valve rotor seals (chromatographic conditions as in Fig. 2)

ments in which isolated rotor seals were immersed in indomethacin solution.

It was found (Table 1) that on incubation of the seals with indomethacin solution the fall in analyte concentration in the supernatant was greater in the vial containing the Vespel rotor seal than both the ones containing the Tefzel rotor seal and stainless steel. Moreover, following removal from the indomethacin solution, washing with water and immersing in mobile phase, it was found (Table 2) that the Vespel rotor seal yielded the most indomethacin. In correlating the results of the initial indomethacin adsorption phase of the experiment with the second desorption phase, it appeared that there had been a lower-than-expected recovery of indomethacin from the Tefzel rotor seal and stainless steel. This apparently anomalous result can be rationalised quite simply. For these weak adsorbers the water-wash step would have caused significant desorption thus leaving less indomethacin to be desorbed by the mobile phase.

Another important finding from these experiments was that there was no significant difference between the results from the Tefzel rotor seal and the stainless-steel outer ring stripped from a rotor seal. This suggests that the Tefzel gives little indomethacin adsorption and that the majority of the indomethacin adsorption in the Tefzel seal case was due to the outer stainless-steel ring, which, of course in situ would not be exposed to analyte. If anything the isolated stainless-steel ring gave greater adsorption than the Tefzel rotor seal. This is in line with the fact that for the isolated ring there would be approximately twice the surface area of stainless steel exposed to the indomethacin solution.

It was also possible to use these indomethacin bulk solution experiments to confirm that the adsorption/desorption on Vespel is a relatively slow process. It proved to be slow enough for an analyte desorption profile (Fig. 10) to be constructed. Slow desorption times such as these account for the previously observed losses in efficiency. Therefore in the interests of efficiency as well as precision and accuracy, when using the peak-compression effect it is important to use a solvent weakly-eluting enough to give peak compression but not so weak that significant adsorption in the injection valve takes place.

4. Conclusions

Although the work described here relates in the main to indomethacin, similar results were obtained for flufenamic acid and a limited number of observations suggested that ibuprofen followed the same pattern. For these NSAID it is apparent that although adsorption in the injection system may take place on

Table 2
Adsorption/desorption of indomethacin on valve rotor seals

	Mass adsorbed (μg)	% Total mass	Mass recovered after desorption (μg)	% Adsorbed mass recovered
Tefzel	1.450	2.8	0.0410	2.8
Vespel	2.650	5.3	1.4900	56.1
Steel ring	1.650	3.3	0.0512	3.1

Experimental conditions as in Table 1.

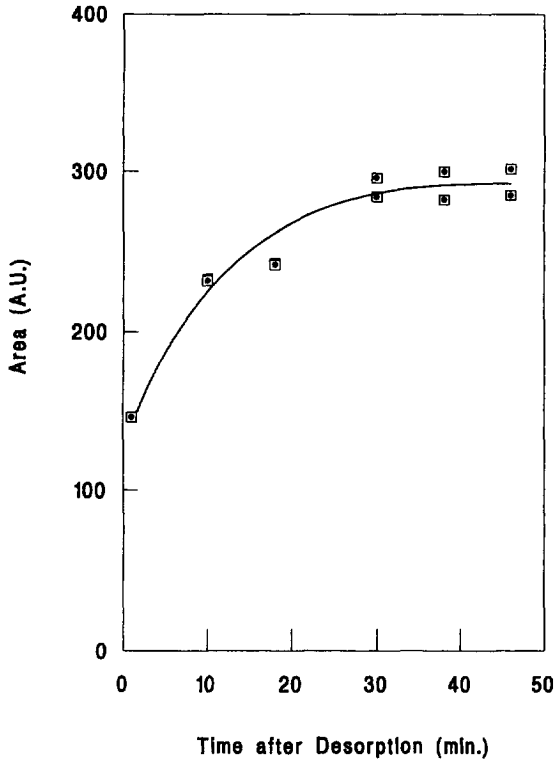


Fig. 10. Desorption of indomethacin with time from a Vespel valve rotor seal which had been incubated with indomethacin solution 1 $\mu g\ ml^{-1}$ in 0.02 *M* phosphate buffer (pH 7.0). Samples of 5 μl injected into a 10-μl sample loop from a solution of mobile phase used to desorb indomethacin. Chromatographic conditions as in Fig. 2.

a variety of surfaces, it arises mainly from interaction with Vespel in rotor seals, whether with the polymer itself or with the graphite impregnated in it. The difficulty in completely avoiding adsorption effects has practical significance with respect to developing quantitative methods based on low-dispersion reversed-phase LC using the peak-compression effect. The solvent used for sample solutions should contain the maximum proportion of organic component that will be sufficient to bring about the desired degree of peak compression. In addition, if it is not possible to avoid the use of valve injectors altogether or to use some means of avoiding loop overfill, then to reduce the inevitable loss of accuracy and precision, the minimum loop overfill volume should be used and that volume should be kept constant for all injections.

Acknowledgments

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