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NICKEL GAS CHROMATOGRAPHIC COLUMNS: AN ALTERNATIVE TO GLASS FOR BIOLOGICAL SAMPLES

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SUMMARY

Nickel tubing may be substituted for glass in the fabrication of gas chromatographic columns for use with samples of biological interest. Comparisons of separations of mixtures of steroids, narcotic alkaloids, phenothiazines, and amphetamines on stainless steel, glass, and nickel packed columns showed little or no observable sample decomposition on glass or nickel as contrasted to complete loss of certain compounds on stainless steel. The nickel columns are easily prepared, durable, economical, and not subject to breakage.

INTRODUCTION

Nearly all gas chromatographic (GC) packed columns currently in use are constructed of either stainless steel or glass tubing. That the choice is shared by these materials indicates that neither possesses all the qualities desired in a chromatographic tubing. Stainless steel is certainly the more easily handled and is quite satisfactory for many analytical applications. Glass, on the other hand, has a less reactive surface which permits separations of labile compounds at high column temperatures; and for this reason, glass is the preferred tubing material for biomedical applications. The surface inertness of glass is perhaps the only property that recommends it in the face of numerous disadvantages. Glass columns are often difficult to connect into the chromatographic system, they lack flexibility and must be formed with precision in order to fit a specific instrument, and they usually require the services of a skilled glass-blower for fabrication which adds to their expense. But these detractions are minor compared to the dismaying fragility of glass which makes changing columns a test of skill for the most experienced chromatographer.

The usual alternatives to glass and stainless steel are, however, even less attractive: copper oxidizes rapidly at elevated temperatures, and decomposition is often severe on copper columns[1]; aluminum has an oxidized surface that is quite active and is difficult to passivate[2]; noble metals such as gold and platinum are far too expen-

sive for general use; and plastics are usually limited to low temperatures. Another possibility was suggested by the introduction of nickel capillary tubing for preparation of high resolution open tubular columns[3]. At least one application of nickel capillary tubing showed that this material could be used in high temperature separation of certain compounds where decomposition on stainless steel had been a problem[4]. Analyses of compounds of biomedical interest, however, are often performed more conveniently on packed columns. Consequently, the extension of nickel tubing to this application was the next logical step.

A comparison of separations of several classes of compounds which experience had shown were subject to decomposition was undertaken using closely matched columns made of stainless steel, glass, and nickel. In these studies nickel columns proved to be surprisingly inert, and yielded chromatographic separations comparable to glass columns.

EXPERIMENTAL

Columns

Nickel columns were prepared from 1/8 in. O.D., 0.020 in. wall, nickel-200 tubing obtained from Handy and Harman (Norristown, Pa., U.S.A.). The tubing was cleaned sequentially with ethyl acetate, methanol and water. The interior surface of the tubing was then etched by filling the tubing with 50% nitric acid and after about 10 min rinsing with water until neutral. This treatment was followed by an acetone rinse, and the tubing was then dried with a stream of air.

The stainless-steel column of tubing of identical dimensions (Handy and Harman) was cleaned in the same manner as the nickel column but with omission of the nitric acid treatment. The glass column was 1/8 in. O.D., 0.08 in. I.D. and was cleaned with ethyl acetate, and toluene. This was followed with a silylation treatment which consisted of filling the column with Tri-Sil (Pierce, Rockford, Ill., U.S.A.), diluted about 1:1 with toluene followed by rinsing with toluene and methanol. All three columns were 6 ft. long and coiled.

The columns were packed with 5% SE-30 on 80–100 mesh Gas-Chrom Q obtained from Supelco (Bellefonte, Pa., U.S.A.). In each case packing was accomplished with light tapping with aspirator vacuum drawn on the column.

Gas chromatograph

A Varian Model 1200 instrument was utilized in this study and was selected because the columns could be installed for on-column injection, and the outlet of the column could be terminated at the jet of the flame ionization detector. Thus any effect of injector, transfer lines, and connectors on the experimental compounds was avoided.

Samples

All sample mixtures were prepared from compounds which had been examined for purity on other chromatographic systems. In each case the compounds were dissolved in redistilled ethyl acetate at 1 mg/ml concentration and 1 μl was injected on the chromatographic columns.

RESULTS AND DISCUSSION

A variety of classes of compounds known to present difficulties in GC analysis were examined on each of the three columns. The stationary phase, SE-30, was not necessarily the optimum for all of the experimental samples. Experience had shown, however, that if decomposition were to occur on a column, the loss of sample, distortion of peak shape, or appearance of extraneous peaks would be most noticeable with this non-polar, methyl silicone polymer.

Exact replication of retention characteristics of the three columns was not attainable even though some of the columns were repacked several times under vary-

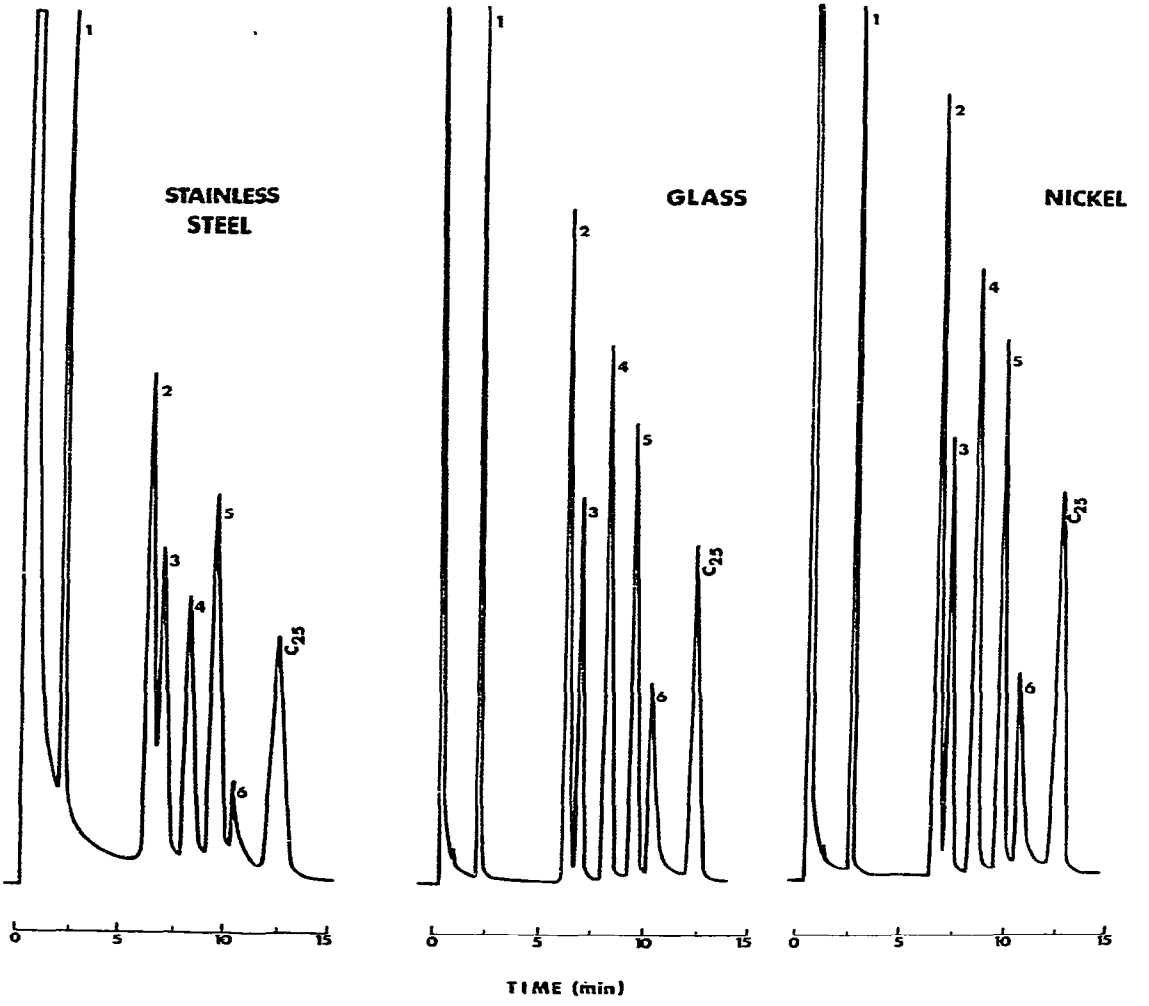

Fig. 1. Separation of a mixture of alkaloids and narcotic analgesics on stainless steel, glass, and nickel packed chromatographic columns. Peaks: 1, meperidine; 2, methadone; 3, cocaine; 4, pentazocine; 5, codeine; 6, morphine; and 7, *n*-pentacosane added as an internal standard. Columns were temperature programmed at 4°/min from 180° to 220°.

ing conditions in an effort to achieve this. Consequently, adjustment of carrier gas flow-rate was used to bring the retention times of test compounds into close agreement on the columns. Column temperature was the same for each column for a given test mixture so that this operating parameter would not be an influence on observed differences.

Fig. 1 shows a comparison of the separation of a mixture of narcotic analgesics on the three columns. The nickel and glass columns are very similar in performance, but some loss of sample has occurred on the stainless-steel column. The peak for morphine is noticeably attenuated, and tailing of the solvent peak is more pronounced on stainless steel which often indicates the presence of breakdown products having lower molecular weights than the parent compounds.

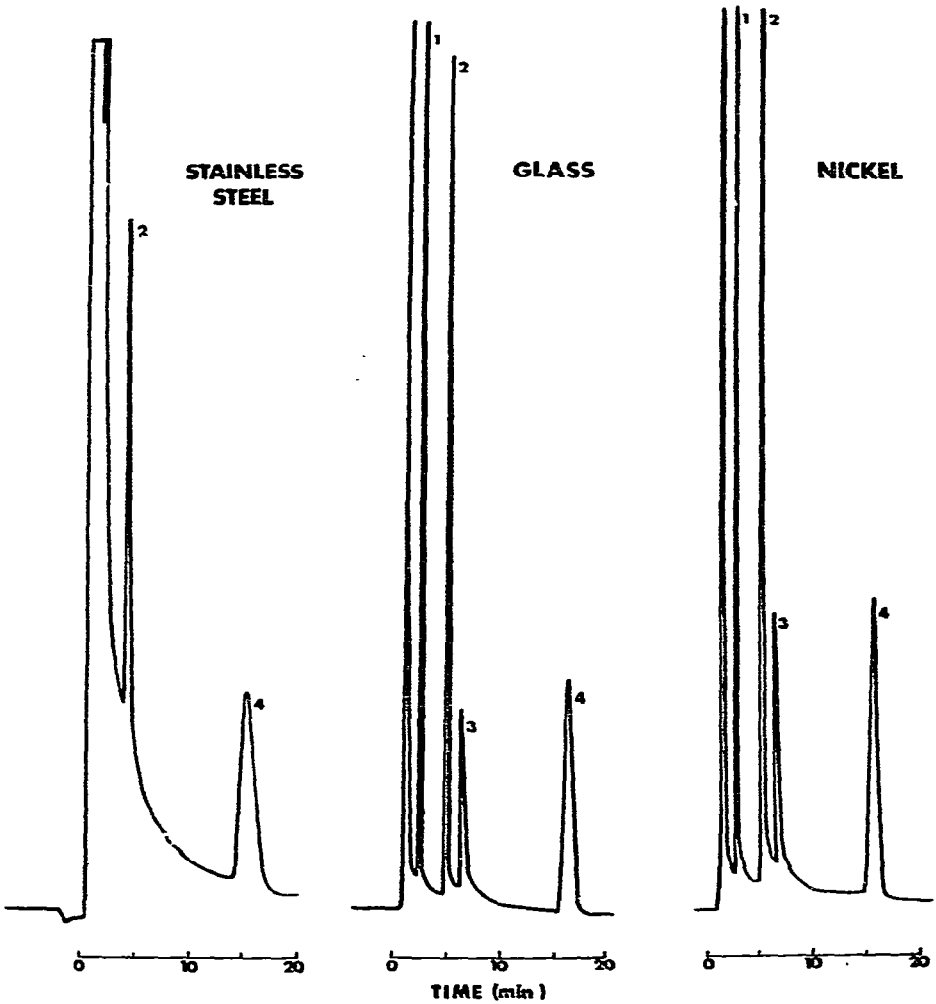

Fig. 2. Comparative chromatograms of a mixture of: 1, methamphetamine; 2, *p*-chloro-N-methamphetamine; 3, *p*-hydroxyamphetamine, and 4, *n*-hexadecane as internal standard. Column temperatures were 120°.

Somewhat more dramatic differences are observed in the analysis of amphetamines as seen in Fig. 2. Pronounced tailing of the solvent peak has all but obscured the methamphetamine peak, and the hydroxyamphetamine peak has disappeared on the stainless-steel column. The chromatograms obtained on glass and nickel are again quite similar, although some peak tailing has occurred. This tailing is not unexpected considering the SE-30 stationary phase employed in these comparisons is not the usual choice for analysis of amines[5].

The phenothiazine drugs constitute a particularly difficult class of compounds for successful GC analysis, because these substances are not only labile but also

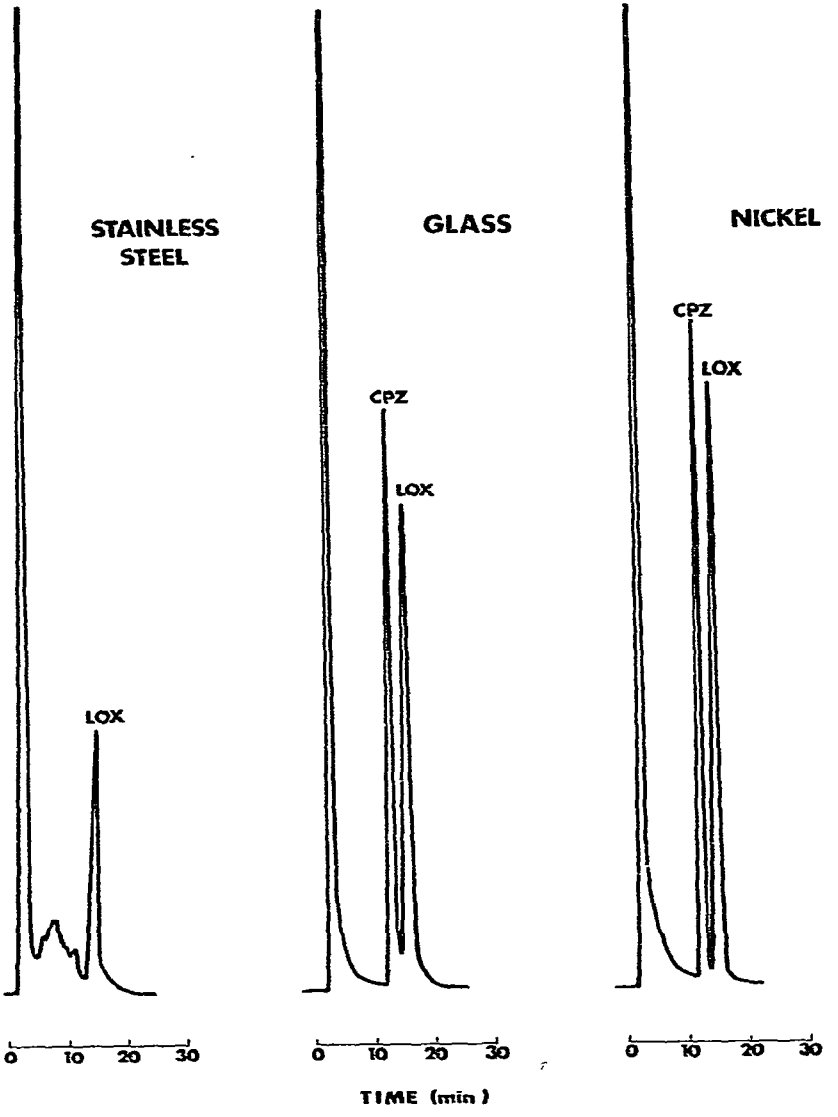

Fig. 3. Separation of chlorpromazine and loxapine at 230° on stainless-steel, glass, and nickel columns.

require high column temperatures for elution in any reasonable time. As can be seen in Fig. 3, chlorpromazine is entirely decomposed on the stainless-steel column and for this reason glass columns have been a necessity in the analysis of chlorpromazine as well as most other psychotropic drugs having similar structures[6]. The nickel column, however, yielded a very acceptable chromatogram, and similar columns have been employed in our laboratories for many months in routine determinations of drug blood levels[7].

While the effect of column material is not as apparent on the separation of the

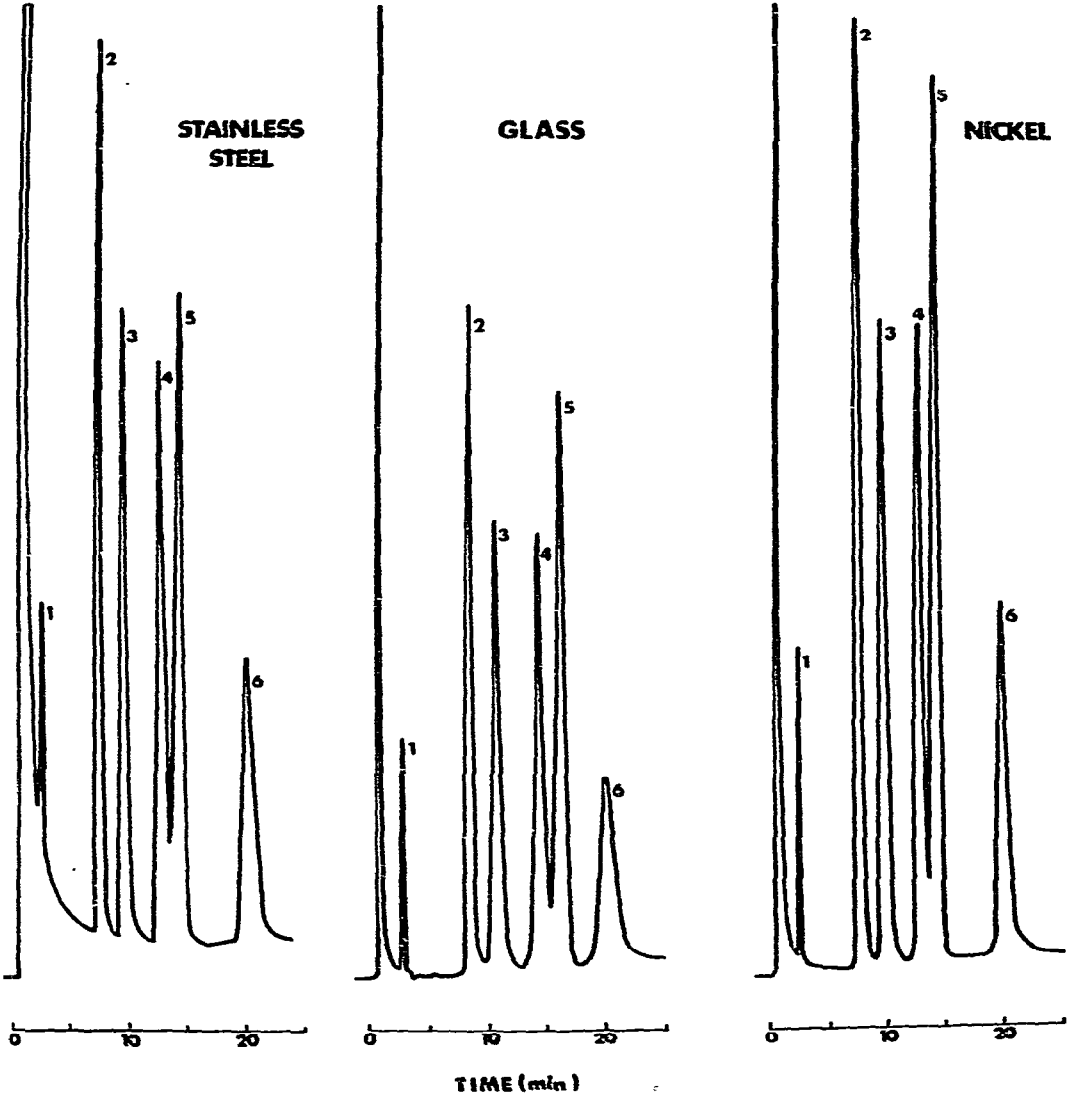

Fig. 4. Comparative separation of a steroid mixture containing: 1, eicosane as internal standard; 2, dehydroepiandrosterone; 3, testosterone; 4, progesterone; 5, 5α-cholestane; and 6, cholesterol. The columns were programmed at 2°/min from 210° to 250°.

steroid mixture shown in Fig. 4, some differences do exist. Again tailing of the solvent front is more noticeable on stainless steel which suggests some decomposition of the sample mixture.

The performance of packed nickel columns may sometimes be improved by silylation which is usually performed by injection of silylating reagents such as Silyl-8 (Pierce) at elevated temperatures, assuming, of course, that the stationary phase is compatible with this treatment. Whether silylation acts on the packing, tubing walls, or both remains to be determined, but the improvement is comparable to that observed in glass columns through similar treatment.

Other classes of compounds which we have chromatographed on nickel column are barbiturates, cannabinoids, catecholamines and catecholamine metabolites, and various perfluorinated ester derivatives used in electron capture detection. Without exception compounds which could be chromatographed on glass columns yielded comparable chromatograms on columns constructed of nickel tubing.

An additional advantage to be gained through the use of these columns is purely economic. Apart from the total lack of breakage that accompanies use of metal columns, the cost of nickel tubing at the time of this report is, measure for measure, about one-tenth that of glass columns obtained from instrument manufacturers or chromatographic supply houses.

Although nickel would appear to serve quite satisfactorily in most applications now requiring glass columns, there is the possibility that only glass will provide the requisite inertness at temperatures above those employed in this study. Studies of metal columns for GC at elevated temperatures are, however, far from being exhausted. Our own experience with metal *versus* glass is such that where performance is comparable, the glass column is relegated to the storage cabinet rather than being used in a working instrument.

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