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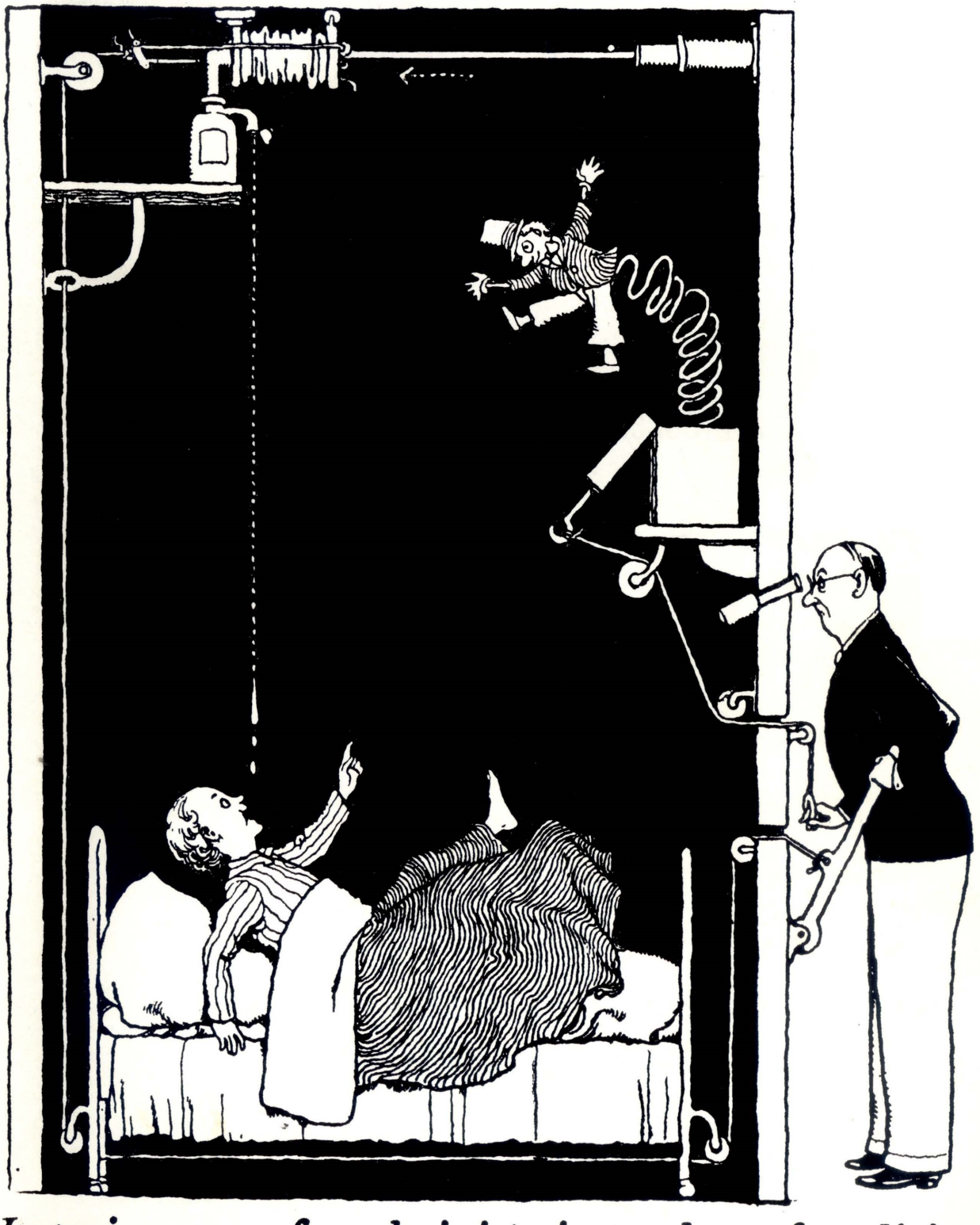


Ingenious ruse for administering a dose of medicine to a boisterous boy

Alfred J. Vella

Fuel mixtures – a forensic question

Gas chromatography is a useful analytical technique that can be taught in the guise of a forensic investigation. Here, a real forensic problem regarding the contamination of fuel is investigated.

Gas chromatography is one of the most frequently used and powerful tools available for separation and analysis, because of its speed, resolving power, and extreme sensitivity. For quantitative work, a calibration curve is invariably required. Intuitively, such curves are expected to be straight lines, but in practice they are usually not. I would like to present a real problem in forensic chemistry that lends itself to a practical exercise in high resolution gas chromatography,[1] involving a nonlinear calibration curve. The reasons for the nonlinearity of the curve are discussed and the experimental curve is modelled on the basis of a simplified treatment of the analytical problem.

The analytical problem

In countries like Malta, where kerosene is sold for domestic heating at subsidised rates well below the price of other fuels, it is illegal to use the material as an automotive fuel. However, the price difference often proves irresistible and people routinely appear in court for possessing mixtures of kerosene and diesel fuel (gas oil). Such mixtures are typically found by the police in the fuel tanks of diesel vehicles – after which the forensic chemist is involved.

The forensic chemist must confirm the presence of kerosene in suspect diesel fuel at concentrations which may vary from 100 per cent to about 5 per cent by volume. Capillary column gas chromatography with flame ionisation detection is the method of choice for such analyses.

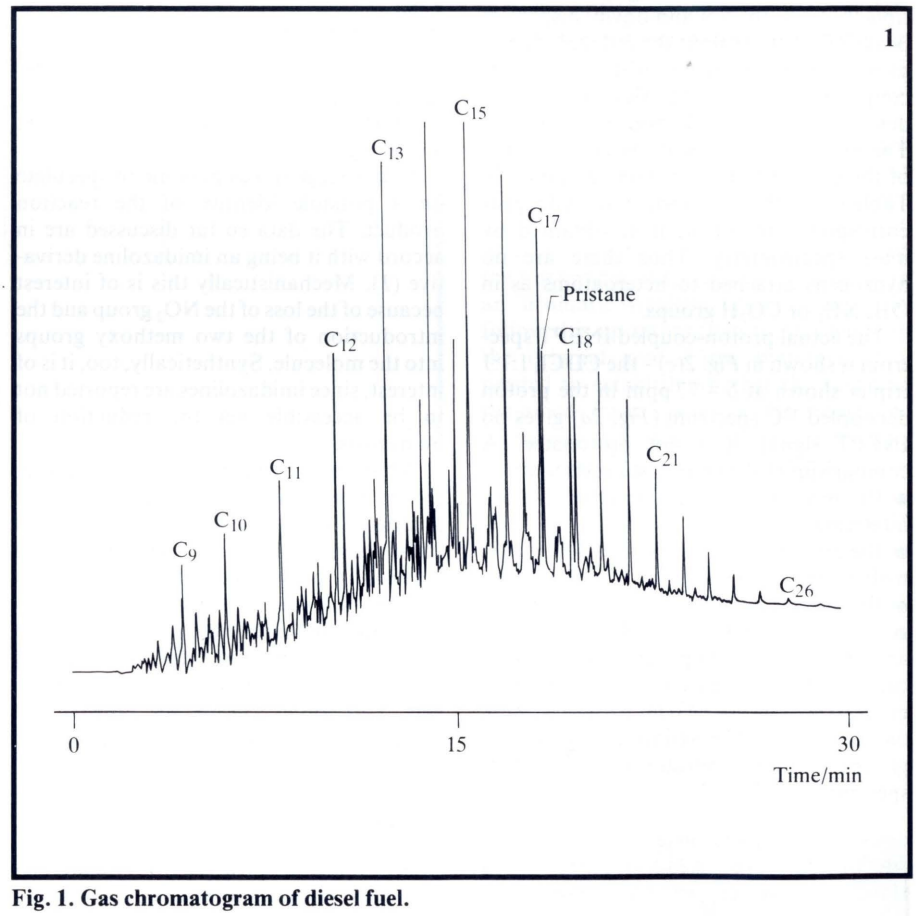


Fig. 1. Gas chromatogram of diesel fuel.

Diesel fuel is a complex mixture of hydrocarbon and non-hydrocarbon substances. In a detailed analysis of the diesel fraction from petroleum, Robinson[2] was able to identify 19 molecular compound types, including saturated acyclic alkanes, mono-, bi- and tricyclic alkanes (naphthenes*), aromatic compounds, including alkylbenzenes, naphthalenes, pyrenes, acenaphthenes and perylenes, as well as non-hydrocarbon compounds such as benzofurans and dibenzothiophenes.

Figure 1 is a gas chromatogram of a sample of diesel fuel obtained by using a capillary column in a programmed temperature run. The major peaks are n-alkanes (C_9

*Naphthene is a term used in petroleum chemistry to denote certain saturated hydrocarbons, specifically five- and six-membered cycloalkanes and their alkyl derivatives.

Table 1. Values of R for standard diesel and kerosene/diesel fuel mixtures.

Kerosene (percentage by volume)	R
0	0.056
5	0.18
15	0.36
30	0.71
50	0.98
70	1.3
80	1.4
95	1.8

through C_{26}); these compounds are accompanied by others, some of which are also fairly abundant. In particular, the chromatogram shows a characteristic baseline 'hump', referred to by petroleum chemists as the 'unresolved complex mixture' (UCM).

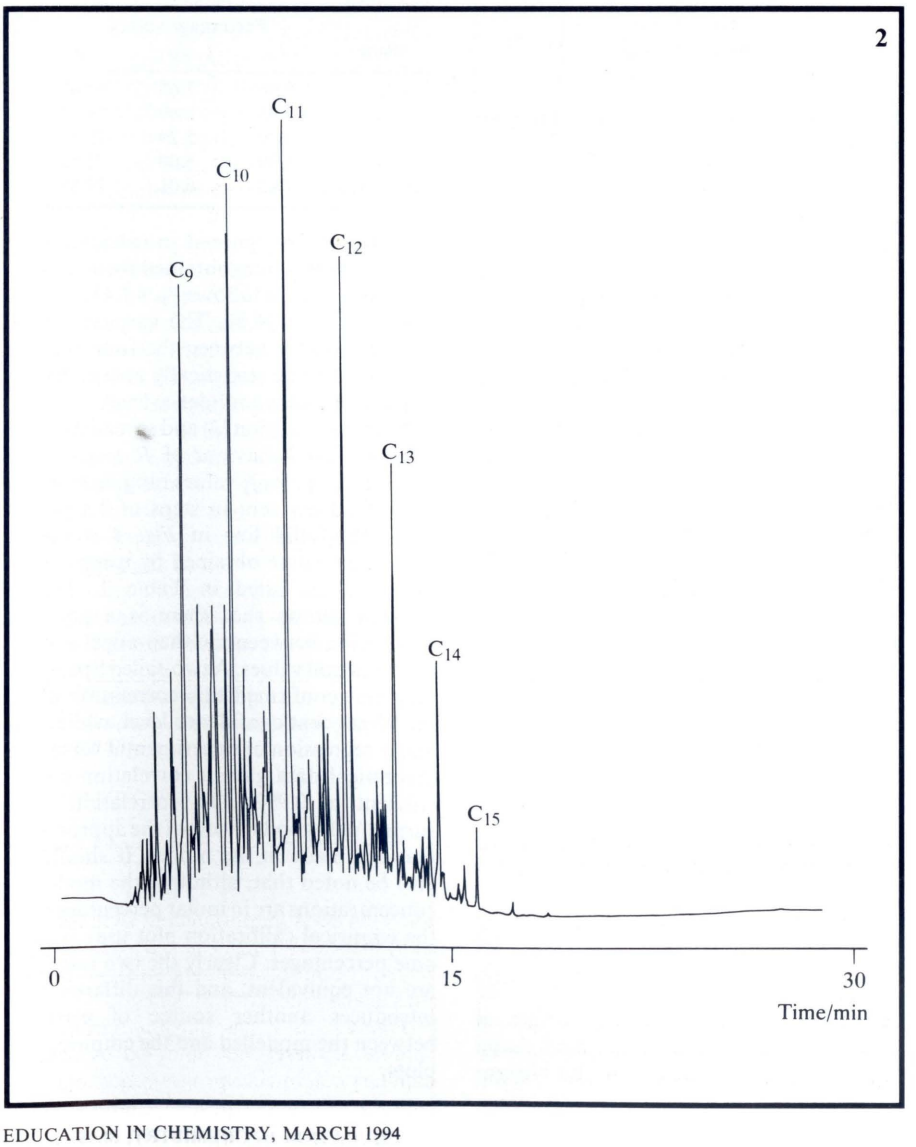
Kerosene is a mixture of compounds, mostly hydrocarbons, dominated by the n-alkane series from nonane to pentadecane, with smaller amounts of aromatic hydrocarbons and naphthenes. Naphthenic acids, phenols and thiophenes are among the non-hydrocarbon compounds in kerosene. *Figure 2* is a gas chromatogram of a sample of kerosene run under the same conditions as used for *Fig. 1*, and showing the n-alkanes as major peaks.

Since all the n-alkanes in kerosene are also found in diesel fuel, a mixture of the two liquids will have an n-alkane profile that is different from that of neat diesel fuel by being richer in the alkanes that elute earliest (*Fig. 3*). A calibration plot can thus be prepared from standard mixtures of kerosene and diesel fuel by using a parameter that serves as a measure of the kerosene-derived alkane content in the hydrocarbon mixture. We can conveniently define a parameter, R, as:

$$R = \log_{10}\left\{\frac{([C_9]+[C_{10}]+[C_{11}]+[C_{12}])}{([C_{17}]+[C_{18}])}\right\} \quad (1)$$

where $[C_i]$ refers to the molar concentration of the n-alkane with carbon number i in the fuel mixture. Nonane, decane, undecane and dodecane occur both in diesel and in kerosene, while heptadecane and octadecane are both practically absent from kerosene. The very large difference in the value of $(C_{17} + C_{18})$ for the two fuels means that we have to adopt a logarithmic parameter for R, since otherwise the fraction would vary from a value of several thousands for kerosene to less than one for pure diesel. It is very easy to locate the peaks corresponding to heptadecane and octadecane in the gas chromatogram, since they are always accompanied by the closely eluting isoprenoid alkanes 2,6,10,14-tetramethylpentadecane (pristane) and 2,6,10,14-tetramethylhexadecane (phytane) respectively (*Fig. 1*).

Fig. 2. Gas chromatogram of kerosene.



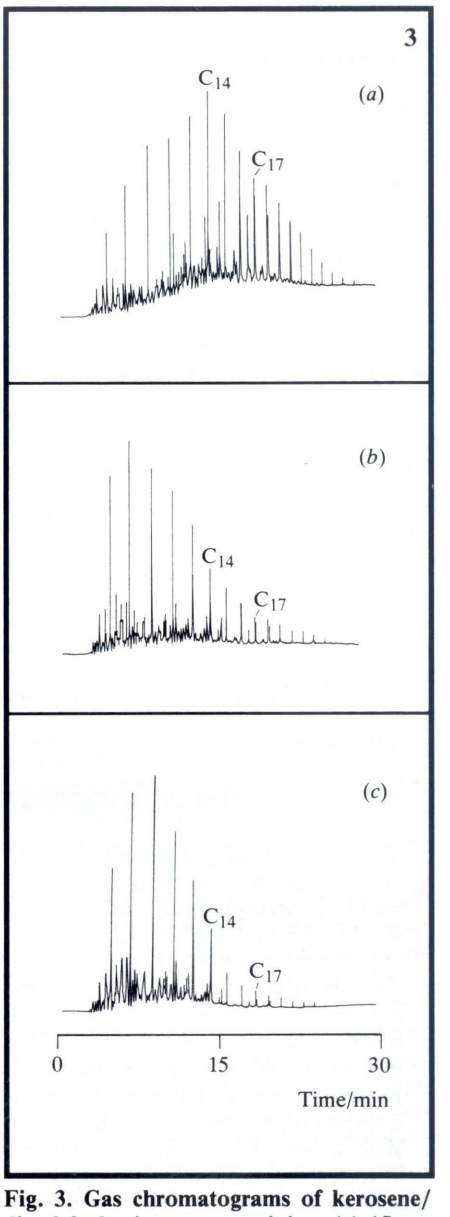


Fig. 3. Gas chromatograms of kerosene/diesel fuel mixtures containing: (*a*) 15 per cent; (*b*) 60 per cent; (*c*) 80 per cent kerosene.

Experimental

Students are presented with exhibit 1, an 'alleged' mixture of kerosene and diesel fuel of unknown composition, and they are also supplied with samples of diesel fuel and kerosene that are 'certified as unadulterated' by the local fuel supplier. About 10 ml of each fuel should be more than sufficient for the analysis. Standard mixtures could be prepared by using a total amount of mixture of 500–1000 μl (or larger volumes if micropipettes are not available). For the calibration curve, the mixture composition can be plotted in terms of volume percentages.

I used a Perkin Elmer model 8600 gas chromatograph fitted with a 25 m long, narrow bore (0.22 mm), fused silica capillary column with polymethylsiloxane as the

bonded phase (*eg* BP1, SGE Australia) for the experiment. The following gas chromatography conditions are suitable for the analysis: helium as the carrier gas at a head pressure of 10 psig (68.95 kPa)†; a flame ionisation detector set at 285 °C and a split/splitless injector set at 280 °C; and a temperature programme of 5 min isothermal at 100 °C, then to 300 °C at 10 °C per minute, then held for 5 min. The total run time is 30 min. A volume of 0.1 μl is injected, with a splitting ratio of about 40. Splitting the injection volume does not have an appreciable effect on the repeatability of the results.

Results and discussion

Table 1 shows experimental values of R obtained for a neat diesel sample and for seven kerosene/diesel mixtures. *Figure 4* shows the experimental points through which an empirical plot is drawn as a dotted line.

For most unadulterated diesel fuels, R is fairly close to zero. As the kerosene content in a diesel fuel increases, so does R, but the variation is not linear. Rather, the calibration plot of R versus the percentage of kerosene starts from a point near the origin, and curves upwards in a nonlinear fashion to approach the 100 per cent value asymptotically, as shown in *Fig. 4*.

Refinery products such as fuels tend to vary in composition. This variation may affect the value of R, especially at the origin of the calibration curve. So, for mixtures containing less than about 5 per cent kerosene in diesel fuel, this technique retains its accuracy only if unadulterated diesel and kerosene samples (which are known to have been used to make the illegal mixture) are available to prepare the standards.

This experiment affords a good opportunity for students to work with nonlinear

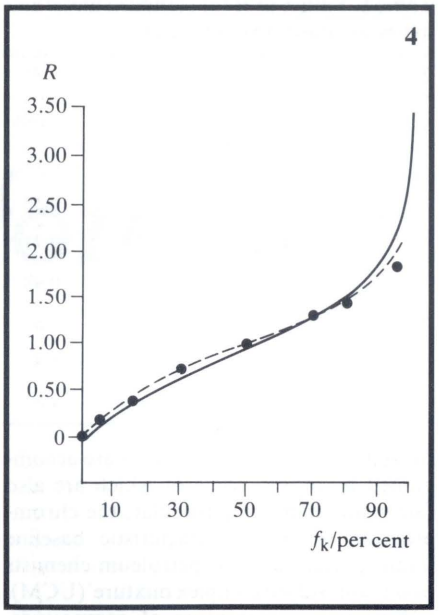


Fig. 4. Calibration curve obtained empirically (broken line) compared with the modelled curve (full line).

†The unit of gauge pressure is psig – *ie* the pressure measured with respect to that of the atmosphere. So 10 psig is 10 psi above the atmospheric pressure. The unit of absolute pressure is psi measured relative to zero pressure.

Modelling the calibration curve

To a first (and, admittedly, crude) approximation, kerosene can be considered as a mixture of the four n-alkanes C_9 through to C_{12}. Diesel fuel can be regarded as consisting of the same four alkanes, mixed with C_{17}, C_{18} and a host of other components, including the other n-alkanes (C_{13}, C_{14}, C_{15}...), the isoprenoids, naphthenes *etc*, which can be regarded collectively as equivalent to a single component M. In addition, we shall assume that the molar abundance of each component, i, is directly proportional to the peak height observed in the chromatogram for that component, h_i, *ie* we assume that the detector response factors for all the compounds in the mixture are equal.

For unadulterated diesel fuel:

$$H_d = h_{9d} + h_{10d} + h_{11d} + h_{12d} + h_{17} + h_{18} + h_M$$

where H_d is the sum of the peak heights due to the components in diesel oil. The subscript d refers to the alkane contribution in diesel oil. Since heptadecane and octadecane are present only in diesel, the subscripts for these alkanes are superfluous. Furthermore, on the basis of the approximation made in the model, any contribution towards the component M from kerosene is disregarded. This strategy, albeit crude, helps to keep the mathematics simple.

For kerosene, similarly:

$$H_k = h_{9k} + h_{10k} + h_{11k} + h_{12k}$$

In terms of mole fractions, f_i, we may write, for diesel fuel:

$$1 = f_{9d} + f_{10d} + f_{11d} + f_{12d} + f_{17} + f_{18} + f_M$$

Multiplying by H_d yields the general expression:

$$H_d = H_d(f_{9d} + f_{10d} + \ldots + f_M)$$

resulting in the series of equalities:

$$h_{9d} = H_d f_{9d}$$
$$h_{10d} = H_d f_{10d}$$
$$etc$$

For kerosene, we can write an analogous expression in terms of mole fractions:

$$1 = f_{9k} + f_{10k} + f_{11k} + f_{12k}$$

It follows that, for any mixture of kerosene and diesel fuel,

$$\begin{aligned} & h_9 + h_{10} + h_{11} + h_{12} \\ &= (h_{9d} + h_{10d} + h_{11d} + h_{12d}) + H_k \\ &= H_d - (h_{17} + h_{18} + h_M) + H_k \\ &= H_d - H_d(f_{17} + f_{18} + f_M) + H_k \end{aligned}$$

Also, for such a mixture,

$$h_{17} + h_{18} = H_d(f_{17} + f_{18})$$

Now, since the molar concentrations are proportional to peak heights, and the proportionality constant is assumed to be common to all hydrocarbons, it follows that:

$$\begin{aligned} R &= \log_{10}[(h_9 + h_{10} + h_{11} + h_{12})/(h_{17} + h_{18})] \\ &= \log_{10}\left\{\frac{[H_d - H_d(f_{17} + f_{18} + f_M) + H_k]}{H_d(f_{17} + f_{18})}\right\} \\ &= \log_{10}\{[1 - (f_{17} + f_{18} + f_M) + r_k]/(f_{17} + f_{18})\} \end{aligned}$$

where $r_k = H_k/H_d = f_k/f_d$, and f_k and f_d are, respectively, the fractions of kerosene and diesel fuel in the mixture. By definition, $f_d = (1 - f_k)$, and so:

$$R = \log_{10}\left[\frac{1 - (f_{17} + f_{18} + f_M) + f_k/(1 - f_k)}{f_{17} + f_{18}}\right] \quad (2)$$

Values for f_{17}, f_{18} and f_M can be determined by performing a number of repetitive runs with unadulterated diesel fuel. Several small peaks in the chromatogram can be ignored in calculating f_M. The mean values obtained from four such runs were as follows: $f_{17} = 7.45$; $f_{18} = 6.01$; and $f_M = 74.89$. The variation of each parameter between the four runs was found to be statistically acceptable at the 95 per cent confidence level.

Table 2. Values of parameters f_{17}, f_{18} and f_M obtained from chromatograms of neat diesel fuel.

Run	Percentage values		
	f_{17}	f_{18}	f_M
1	9.96	7.67	67.03
2	7.05	6.09	79.17
3	6.79	5.29	75.75
4	5.99	5.00	77.62
Mean values	7.45	6.01	74.89

By using equation (*2*) and spreadsheet software, the behaviour of R versus f_k was simulated for f_k values ranging from 0 to 100.0 per cent in steps of 0.1 per cent. The solid line in *Fig. 4* is the theoretical curve obtained by using the mean values listed in Table 2. The diagram shows that there is a good correlation between the theoretical and experimental values. A two-tailed Spearman test confirmed this correlation at the 95 per cent confidence level, while a linear regression of experimental versus theoretical data gave a correlation coefficient of 0.992. The correlation is surprisingly good in view of the approximations made in the model. It should also be noted that, although the model concentrations are in molar percentages, the empirical calibration plot uses volume percentages. Clearly the two ratios are not equivalent, and this difference introduces another source of error between the modelled and the empirical plots.

calibration plots. In most chemical analyses we tend to expect, and delight in obtaining, linear variations of a measured parameter with concentration. In this expectation we are obviously strongly conditioned by our experiences in spectrophotometric work, and Beer's law in particular. Indeed, the advantages of a linear calibration line need hardly be stressed. This experiment is thus useful for two main reasons: (*i*) it illustrates that calibration plots can deviate from linearity for reasons that are not related to chemical or physical interferences; and (*ii*) it shows that, in most cases, reliable results can be obtained even from curvilinear plots, provided that the number of standard points is suitably large in the region of interest.

The opportunity to work with a nonlinear calibration plot should be exploited to the full. For this purpose, in addition to the experimental work, students should be invited to model mathematically the unusual shape of the curve. Besides being the most effective way of rationalising the plot, this exercise also gives the analysts something to do during the chromatographic runs. An attempt at modelling the calibration curve is shown in the Box.

Conclusion

This article presents an analytical problem taken from the real world of forensic chemistry, with the added advantage that the materials required are of relatively low toxicity and are readily available. The analysis presents an unusual situation in which the calibration curve is nonlinear for reasons related to the nature of the analyte rather than instrumental, matrix or other such effects. It will be easy for students to appreciate why such a curve will need more frequent data points for reliable interpolation. In addition, the experiment should provoke students to reflect on the complex chemical nature of such commonplace commodities as kerosene and diesel fuel.

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continued from page 39.

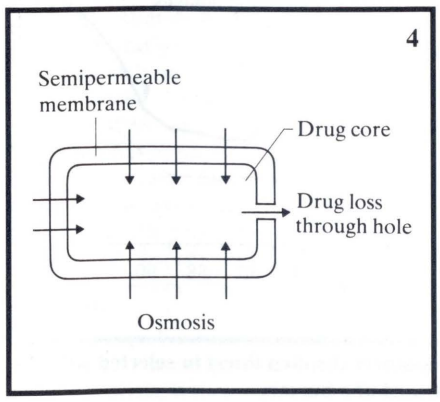
The drug can be released at different times by using granules with several coatings of different thicknesses within the same drug capsule.

Continus system. In the Continus controlled release system, drug release depends upon the dissolution of a long chain aliphatic alcohol, and the diffusion of the active component through a hydrated hydroxyalkyl cellulose. The system is simple but effective: the drug particles are first coated with a hydrophilic cellulose layer that has been partly hydrated; they are then dried and granulated before being incorporated into a molten hydrophobic higher aliphatic alcohol. On cooling, the solid is granulated and the granules compressed to make the final tablet.[1,5]

By using this system, several parameters can be altered to vary the release rate of the drug. They include:

- increasing the thickness of the cellulose coating, thereby increasing the time it takes for the drug to diffuse through it;
- increasing the degree of hydration of the cellulose – the drug will diffuse faster through a more hydrated cellulose, because long polymer chains are more flexible when hydrated, causing the pores in the cellulose network to open up and allow the drugs to diffuse through.
- increasing the amount of hydrophobic alcohol in the formulation – if there is more alcohol there, it will take the gastric juices longer to dissolve the alcohol and expose the cellulose coated drug;
- increasing the length of the alkyl chain of the alcohol, so reducing its solubility.

Fig. 4. The osmotic pump.



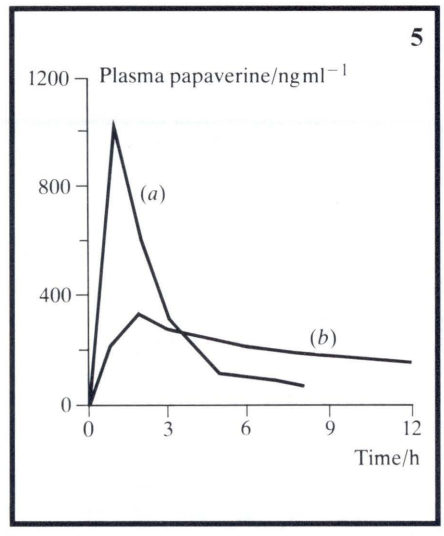


Fig. 5. Papaverine is a non-specific smooth muscle relaxant with a therapeutic range of 150–300 ng ml^{-1}. This graph shows the mean plasma concentrations of the drug after the administration of 300 mg by (*a*) conventional and (*b*) controlled release formulations.[7]

Many drugs can be formulated in the Continus system – an example is Uniphyl, a theophylline bronchodilator, taken once daily.

By varying the exact composition of the tablet it is possible to design systems that will release the drug *in vivo* over periods of 1–12 h, so producing whatever plasma profile is required (*Fig. 5*). An advantage of mechanisms like the Continus system is that the rate of drug release is not affected by the patient's eating pattern.[6]

Disadvantages

It would be foolish to think that there are no disadvantages with controlled release systems.[3,4] Compared with single dose treatments, it is expensive to produce controlled or sustained release formulations. They tend to contain more of the active drug than single doses because of the length of time over which they release it, and if the release mechanism fails, the patient could receive either no dose or far too much, with all the potential toxic side effects. Any gastrointestinal blockage could also cause problems, because the drug would be released over a small area, leading to a localised high concentration that might damage surrounding tissue.

Although one of the benefits of sustained release mechanisms has been to encourage greater patient compliance, there is a greater loss of efficacy if a controlled release dose is missed, compared with a missed dose of the conventional formulation. Consequently, the drugs that are suited to sustained release formulations must be considered extremely carefully – the benefits of the continuous release system must outweigh the resulting cost and possible side effects if the drug is not delivered precisely as it is intended.

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