Infrared Pseudo Matrix Isolation Spectroscopy: Analysis of Gas Mixtures

Abstract. *A new low-temperature technique provides a practical and sensitive method of infrared quantitative analysis of all infrared-absorbing gases and volatile liquids. Because pseudo matrix isolation is suitable for qualitative identifications also, it provides a tool for analysis of complex gas mixtures.*

Recently reported (*1*) is a new technique for low-temperature condensation of gases which provides a general method of infrared qualitative analysis of multicomponent gaseous mixtures; no preanalytical separation of mixture components is necessary, and the method can differentiate between structurally similar molecules. In principle, the method is an adaptation of the matrix isolation technique (*2*) to the problem of spectrochemical analysis. While it retains many salient features of conventional matrix isolation, pseudo matrix isolation (PMI) replaces the slow continuous condensation of highly dilute inert gaseous mixtures, characteristic of the conventional method, with a carefully controlled pulsed deposition of only moderately dilute (1-percent) mixtures. The method is rapid (*3*); only minutes are required for complete preparation of samples. We have studied a broad range of chemical compounds including alkanes, simple olefins, dienes, alkynes, ethers, aldehydes, ketones, aromatics, and inorganic oxides and sulfides (*4a*).

Very recent work indicates that the PMI technique provides also a general and practical method of infrared quantitative analysis evidently for all infrared-absorbing gases and volatile liquids (that is, all gaseous molecules except homonuclear diatomics). Every chemical compound examined has yielded a linear Beer's law plot whether the molecule was polar, nonpolar, large (dimethyl propane), or small (methane). Again, preanalytical separation of mixture components is unnecessary. Provided successful qualitative study of a gaseous mixture is indicated, quantitative analysis may follow directly. Spectral analysis consists of frequency measurements for qualitative analysis, and peak absorbance measurements of selected bands for quantitative studies.

The spectroscopy can be performed with any of many conventional infrared instruments, although a spectral slit of 1 to 2 cm^{-1} over the spectral range of interest, 400 to 4000 cm^{-1}, is optimal. Over this range, certain frequency intervals are of limited value for qualitative work (*4b*): for example, the regions 2800 to 3000 cm^{-1} and 1350 to 1500 cm^{-1} are severely overcrowded by a superabundance of C–H stretching and deformation fundamentals.

Certain restrictions on the analytical procedure, regarding technique and apparatus, apply when quantitative studies are planned; they will be detailed elsewhere (*4b*). I now report recent work indicating that this method of cryogenic pulsed deposition opens a new area of application for infrared spectroscopy. This development is significant because of the wide availability of infrared equipment, the commercial availability of cryogenic apparatus (especially Joule-Thomsen refrigerators), and the need in fundamental and applied research for *general* analytical tools for analysis of gases.

Gas analysis is devilishly evasive. Even though gas-phase spectra of different gases are always themselves distinct, serious difficulties preclude the use of gas-phase spectroscopy for analysis of multicomponent mixtures (*1*, *5*, *6*). Only mass spectrometry is fully able to handle analysis of mixtures, but mass-spectral data are complex and often require correspondingly complex analysis. Gas chromatography, a technique that shows remarkable selectivity, requires preanalytical separations to the level of classes of mixture components; alternatively, mixtures must be chromatographed repeatedly or serially on a collection of columns designed to distinguish between chemical classes. One area in which gas chromatography is seriously limited is analysis of molecular isotopes (*7*, *8*). Pseudo matrix isolation provides not only a general analytical tool for qualitative and quantitative gas analysis but also a method for distinguishing between molecular isotopes (*5*). From a vibrational viewpoint, changes in atomic mass are equivalent to changes in molecular structure; thus distinct molecular isotopes act as distinct molecules and submit to infrared spectrochemical analysis.

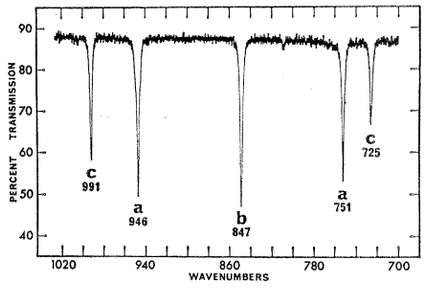


Fig. 1. Infrared PMI spectrum, 20°K, of a mixture of d_2-ethylenes at 1 percent in nitrogen (700 to 1025 cm^{-1}). (*a*) (1,1) Ethylene-d_2; (*b*) *cis*-(1,2) ethylene-d_2; (*c*) *trans*-(1,2) ethylene-d_2. Twelve micromoles of ethylene mixture were deposited. Spectral slit, about 0.9 cm^{-1}.

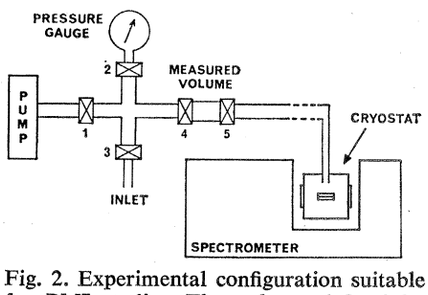
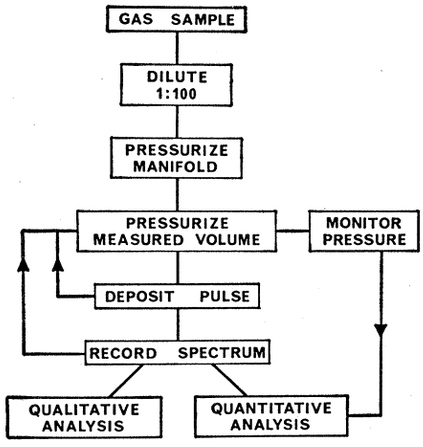


Fig. 2. Experimental configuration suitable for PMI studies. The volume defined by valves 4 and 5 should measure 15 to 16 ml. A glass line with stopcocks is suitable.

The properties of PMI spectra have been described (*1*). Briefly, rotational structure is frozen out, and the matrix dispersion, which results from the *controlled* pulse deposition of a 1-percent solution of reagent mixture in nitrogen on a 20°K halide substrate, suffices to keep intermolecular interactions small. The latter factor rules out direct condensation of undiluted gas mixtures as a suitable sampling technique (*1*, *9*). The cooperative effects of low temperature and matrix dispersion keep the bands narrow (2- to 6-cm^{-1} half-height bandwidths), the spectra simple, and the frequencies reproducible within 1 cm^{-1}. Figure 1 demonstrates the selectivity of the method; it shows a PMI spectrum of a mixture of isotopic d_2-ethylenes (700 to 1025 cm^{-1}). The spectrum resulted from condensation on a clean 20°K CsI window of three 0.46-mmole pulses of a 1-percent solution of the ethylene mixture in nitrogen. Bandwidths of 2 cm^{-1} are observed. The three molecules can be readily detected in the presence of one another, and large disparities in relative concentrations can be measured. Equivalent distinguishability with gas chromatography has not been demonstrated (*7*).

Figure 2 shows schematically an experimental configuration suitable for pulse deposition. The central manifold is connected to the pumps by way of high-vacuum valve 1, to a pressure

gauge (0 to 760 torr) by way of valve 2, to the sample inlet by way of valve 3, and to a measured volume defined by valves 4 and 5. The stepwise procedure from dilution (with nitrogen) of an initial gaseous mixture to spectrum analysis is as follows:



Altering of sample size during an analysis is trivial; one simply causes additional pulses of diluted gaseous mixture to impinge on the cold window by manipulating valves 4 and 5 (and perhaps 2). Just this technique is followed for definition of the PMI Beer's law coefficients for a molecule (*10*). Typical Beer's law plots are shown in Fig. 3. Standard deviations from linearity (least-squares straight lines have been fitted to the data) are characteristically 0.003 to 0.006 unit of optical density. Table 1 collects some of the effective absorption coefficients that I have measured; they are given in terms of both liter per mole-centimeter and micromole. The latter rendition is, apart from a small additive factor, the direct ratio of a measured absorbance to the number of micromoles of reagent gas that passed through the deposition port of the cryostat during sample preparation (*11*).

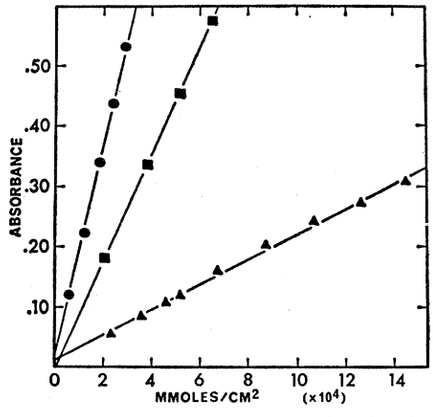


Fig. 3. A PMI Beer's law plot for the d_2-ethylenes. Symbols: ●, *cis*-(1,2) ethylene-d_2 (847 cm^{-1}); ■, (1,1) ethylene-d_2 (751 cm^{-1}); ▲, *trans*-(1,2) ethylene-d_2 (1295 cm^{-1}). The pulses of gas impinge on a substrate having an effective cross section of 19.5 cm^2. The orifice through which the gas streams is an unworked length of Pyrex tubing, 5 mm in internal diameter.

Table 1. Measured PMI Beer's law coefficients. Data for deuterated methanes and deuterated ethylenes have been reported (*5*).

Molecule	ν (cm^{-1})	$e \times 10^{3}$*	E (μm^{-1})†	d (abs)†	s‡	Detectivity (μm)§
Allene	1956	.281	.0144	.0176	.004(9)	0.9
Ethylene	947	.561	.0288	.0048	.001(3)	.9
	1439	.301	.0154	.0138	.002(3)	1.0
Methane	1305	.275	.0141	.0060	.007(13)	1.7
	3025	.106	.0055	.0037	.001(5)	4.8
Methyl ether	1168	.761	.0390	.0115	.006(7)	0.5
Neopentane	1256	.098	.0050	.0129	.003(10)	3.4
	1366	.237	.0121	.0260	.002(9)	0.3
	1470	.131	.0067	.0154	.003(10)	2.2
	1486	.081	.0042	.0225	.004(10)	1.8
Propionaldehyde	1741	.582	.0298	.0230	.002(9)	0.2

* Molar extinction coefficient (liter/mole-centimeter) derived from E. The appropriate unit of concentration is millimole per square centimeter. The substrate cross section was 19.5 cm^2. † Beer's law coefficients: absorbance $= E \times$ (micromoles deposited) $+ d$. ‡ Standard deviation from linearity in absorbance units; numbers of measurements appear in parentheses. § Computed from columns 4 and 5, detectivity is given as the number of micromoles producing an absorption of 0.03 unit of optical density (about 7 percent).

There is no apparent systematic error in the deviations from the origin (Fig. 3); they are reported for other cases in Table 1; in fact it is not clear that the deviations are statistically significant. It is clear though that statistically significant curvature appears in the Beer's law data for the 1295-cm^{-1} band of *trans*-ethylene-d_2 (Fig. 3). These data represent the most severe example of curvature encountered; no other measurements show such apparent nonlinearity. For the case of *trans*-ethylene-d_2, the nonlinearity arises from use of an insufficiently narrow spectral slit for measurement of the intensity of an unusually narrow band. The half-height width for the 1295-cm^{-1} band of *trans*-ethylene-d_2 is less than 1.5 cm^{-1}. Because so narrow a band is exceptional, this kind of photometric error is not a recurring problem. Other factors (*4, 5*) may contribute to PMI Beer's law nonlinearity.

Using parameters from Table 1 I have analyzed several mixtures of gases. One prepared from dimethyl ether (31.0 percent), neopentane (34.4 percent), and air (34.6 percent) was analyzed, with the air disregarded, to have a relative dimethyl ether:neopentane concentration of 46.3 percent. The relative concentration by preparation was 47.4 percent. Nineteen micromoles of mixture were used for the analysis. A mixture of d_2-ethylenes [41.2 percent (1,1), 24.3 percent *cis*-(1,2), 34.1 percent *trans*-(1,2)] was analyzed as 45.0 percent (1, 1), 21.4 percent *cis*, and 33.6 percent *trans*. Errors in quantitative handling of gases currently amount to a few percent, and the spectrometric quantitation is probably accurate within about 5 percent. Note however that, in collection of Beer's law data by repeated pulse deposition, errors in sample preparation bear only on the absolute values of the deduced parameters. The almost strict linearity observed bespeaks precise quantitation. Thus improvement in accuracy is a technical problem.

The spectral simplicity characteristic of PMI infrared spectra accounts for the intrinsically high sensitivity of the method. Whereas the spectral distribution for fundamental absorptions is broad in gas-phase spectra, in PMI spectra all the vibrational oscillator strength for a given fundamental falls within a narrow range of frequencies; peak absorptivities are thus enhanced. The micromolar sensitivity now current (Table 1) (*12*) can be improved substantially by straightforward modification of the spectrometric equipment.

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References and Notes

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2. G. C. Pimentel, *Spectrochim. Acta* **12**, 94 (1958).
3. The conventional matrix method attempts to isolate potentially reactive species in an inert low-temperature lattice or glass. Dilutions of reagent in matrix gas greater than 1000-fold are not uncommon. Because condensation is very slow, sample-preparation time is measured in hours.
4a. M. M. Rochkind and R. V. Albarino, in preparation.
4b. M. M. Rochkind, in preparation.
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10. The sticking coefficient for condensation is very sensitive to alterations in the experimental apparatus; each laboratory must determine how coefficients, measured on its apparatus, scale relative to other reported values.
11. Because the sticking coefficient for condensation is always less than unity, a measured PMI Beer's law parameter always represents a lower bound on the absolute value.
12. The volume of 0.5 μmole of gas is equivalent to about 0.01 cm^3 filled to atmospheric pressure at room temperature.
13. I thank R. V. Albarino for technical assistance.

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Streptomycin Resistance Mutation in Escherichia coli: Altered Ribosomal Protein

Abstract. *Reconstitution of 30S ribosomal particles was performed with 16S ribosomal RNA, "core" proteins, and "split" proteins from 30S particles derived from streptomycin-sensitive and streptomycin-resistant* Escherichia coli *cells in various combinations. Analysis of streptomycin sensitivity of the reconstituted particles has shown that the alteration induced by the resistance mutation resides in the core proteins, and not in the RNA or in the split proteins of the 30S particles.*

A combination of genetic and biochemical methods should be useful in the study of complex cellular organelles such as ribosomes. However, no ribosome mutation has yet been discovered where a functional alteration is correlated to a structural alteration in an obvious way (for a review, see *1*). The most extensively studied ribosomal mutation is concerned with streptomycin (Sm) resistance in *Escherichia coli*. Streptomycin inhibits polypeptide synthesis in vitro (*2*), and the site of its action is believed to be the ribosome (*3*). Thus, polypeptide synthesis of ribosomes from an Sm-resistant mutant is resistant to Sm, whereas that by ribosomes from a wild-type Sm-sensitive strain is sensitive to Sm (*2*). Furthermore, the mutational alteration which leads to Sm resistance is associated with an alteration in the 30*S* ribosomal subunit (*4*). Previous studies (*5, 6*) have shown that the alteration is in the 23*S* "core" portion of the 30*S* particle, and not in the rest of the proteins (split proteins from the 30*S* particles, designated as SP30). Although the altered component was thought to be a protein, no observable alteration had been detected in the ribosomal proteins of appropriate mutants (*1*). Thus, the possibility had not been excluded that the alteration is in the 16*S* ribosomal RNA.

We have achieved reconstitution of functionally active 30*S* ribosomal particles from free 16*S* ribosomal RNA, and the mixture of ribosomal proteins obtained from 30*S* particles, in the following way (*7*). (i) The 23*S* core particles and SP30 proteins were prepared by centrifugation of purified 30*S* particles in 5*M* CsCl (*8*). The SP30 proteins (about 40 percent of the total 30*S* ribosomal proteins) were recovered from the top of the gradient, and the 23*S* core particles were recovered from the band near the middle of the gradient. (ii) The 16*S* ribosomal RNA was prepared from 23*S* core particles by treatment with phenol. (iii) Proteins (CP30) from 23*S* core particles were prepared by treatment of the particles with an equal volume of a solution consisting of 8*M* urea and 4*M* LiCl. (iv) The 16*S* RNA, CP30 proteins, and SP30 proteins were mixed under controlled conditions and dialyzed, and the reconstituted 30*S* particles were recovered by centrifugation. Using this reconstitution system, we now show that the alteration induced by the Sm-resistance mutation resides in the CP30 proteins and not in the 16*S* ribosomal RNA.

An Sm-sensitive strain *Escherichia coli* Q13 and an Sm-resistant mutant derived from it were used (*5*). Both 30*S* and 50*S* ribosomal particles were prepared from these strains and purified (*9*); 16*S* RNA, CP30 proteins, and SP30 proteins were prepared from 30*S* particles from both the Sm-sensitive and the Sm-resistant strains. Both SP30 and CP30 protein fractions were essentially devoid of RNA (less than 2 percent), and the 16*S* RNA preparations contained almost no protein (less than 0.6 percent). The 30*S* particles were reconstituted by the mixing of 16*S* RNA, CP30 proteins, and SP30 proteins in eight different combinations (Table 1). The resultant particles were recovered by centrifugation, and their sensitivity to Sm was tested by following polyuridylic acid–directed phenylalanine incorporation (*10*) in the system containing 50*S* particles from the Sm-sensitive strain (*11, 12*).

The activity of the reconstituted 30*S* particles assayed in the absence of Sm was very high in every case and nearly the same as the activity of native 30*S* particles (Table 1). Also, Sm inhibited the incorporation only when the reconstituted 30*S* particles contained the CP30 proteins derived from sensitive cells, and the degree of inhibition was about the same as that found with sensitive native 30*S* particles. The origin of the SP30 proteins did not show any correlation with the Sm-sensitivity of the reconstituted particles, confirming previous results (*5, 6*). Similarly, the origin of 16*S* RNA did not affect the sensitivity of the reconstituted particles to Sm. Thus, the alteration induced by the Sm-resistance mutation resides in the CP30 proteins and not in the 16*S* ribosomal RNA.

Earlier studies have demonstrated that

Table 1. Sensitivity to streptomycin (Sm) of 30*S* ribosomal particles reconstituted from 16*S* RNA, CP30 proteins, and SP30 proteins. The origin of the components is described in Table 1. The letters r or s indicate that they are derived from Sm-resistant or Sm-sensitive cells, respectively. The reconstituted particles or control undissociated 30*S* particles (60 μg) were assayed for their activity in polyuridylic acid–directed incorporation of phenylalanine in the presence of 50 *S* particles (120 μg) from Sm-sensitive cells and, when indicated, in the presence of Sm (5×10^{-5} mole/liter). The Sm was mixed with ribosomes before the addition of other components. Incorporation was assayed as described (*9*).

Reconstituted "30*S*"			Control 30*S*	Incorporation activity (count/min)		Inhibition by Sm (%)
16*S* RNA	CP30	SP30		− Sm	+ Sm	
			s	11063	7042	36
			r	13174	12995	1.4
s	s	s		10498	6979	34
s	s	r		11904	7889	34
s	r	s		9898	9471	4.3
s	r	r		12037	11921	1.0
r	s	s		11112	7208	37
r	s	r		13098	8486	35
r	r	s		10695	10615	0.6
r	r	r		12491	12579	0 (−0.7)