

# Monochromator Wavelength Calibration Standards Extending into the Near-Infrared Using Second- and Third-Order Emission Lines from Mercury Vapor Lamps

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The emission spectra of four mercury vapor lamps are used to obtain wavelength calibration curves for the double-grating emission monochromator of a spectrofluorimeter. The use of second- and third-order diffraction lines and emission lines from the argon carrier gas provides a rich spectrum, which extends well into the near-infrared spectral region and produces an improved calibration curve. More than 60 emission lines are listed between 250 and 900 nm, which is sufficient to produce an extremely accurate monochromator calibration. Additional second- and third-order lines can be used to even longer wavelengths (>1200 nm). The effectiveness of three scattering surfaces is compared.

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**KEY WORDS:** Monochromator; calibration; mercury; argon; near-infrared.

## INTRODUCTION

The use of mercury vapor lamps as wavelength calibration standards is a common practice in both fluorescence and atomic spectroscopy and is described in instrumental technical manuals [1] and reference texts [2–5]. Two recent papers present highly precise measurements of the wavelengths [6] and intensities [7] of the spectral lines emitted from a mercury lamp for monochromator calibration. The majority of the current literature pertaining to the use of mercury lamps for calibration, however, is oriented toward atomic emission rather than fluorescence spectroscopy and has focused primarily on the UV and blue spectral regions. Typically, only 10 to 12 mercury emission lines, ranging from 254 to 597 nm, are suggested as useful standards, with only three to four lines above 400 nm. Although these bright lines are easily

observed, they provide a sparse set of calibration lines for fluorescence spectroscopy, as Stokes shifts force the emission of many fluorophores into the midvisible or red spectral regions. Further, a great deal of recent activity has extended interest to even longer wavelengths with red and near-infrared emitting sensors and probe molecules (e.g., cyanines, oxazines, and phthalocyanines) [8]. Clearly, the primary mercury emission lines are inadequate for broadband monochromator calibration, especially in the increasingly important 500- to 1100-nm region.

Fortunately, the spectra which are actually observed from low-pressure mercury lamps on a grating instrument are much richer than indicated earlier, and several emission or apparent emission lines exist that extend calibration standards well above 600 nm into the near-infrared. These additional lines arise both from the higher (second- and third-) order diffraction of primary mercury lines and from the line emission of the lamp's standard argon carrier gas [9]. Argon has almost no emission at wavelengths below 700 nm but has several strong emission lines in the 700- to 1100-nm range. The combination of these

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two sets of additional lines provides a rich spectrum for monochromator calibration across the entire range of interest for fluorescence spectroscopy. While the use of higher-order lines for monochromator calibrations is used by at least one fluorimeter manufacturer (Perkin Elmer Corp.), the method seems not to be widely known by the fluorescence community.

Described here is a procedure for using mercury lamps as wavelength calibration standards for the 250- to >900-nm range. A catalog of useful emission lines in this range is presented, and the use of filters to differentiate between diffraction orders is discussed. The method is simple and can be accomplished effectively with inexpensive lamps and other materials that are standard equipment in most laboratories.

## EQUIPMENT

*Lamps.* The four lamps were all readily available low-pressure mercury arc lamps with argon carrier gas in a quartz or glass envelope. Relative emission line intensities depend on the temperature, pressure, and other lamp parameters including the envelope material and additional self-contained filters. Consequently, the number and intensity of available lines varies greatly among sources, with the largest number of lines typically coming from the more expensive lamps. However, since the line emission wavelengths are invariant, all could be used for accurate monochromator calibrations.

*Lamp A.* This lamp was a "pen lamp" with a quartz envelope (Oriental Model 6035). The lamp is powered by an external power supply (Ultraviolet Products Inc., Model SCT1). This lamp emits extremely bright 254-nm radiation and must be used only with suitable eye protection. This is the most expensive of the lamps discussed here.

*Lamps B and C.* This was a dual-bulb hand-held UV lamp used for viewing fluorescent-labeled TLC plates (Mineralight Model UVGL-25, manufactured by UVP). This lamp has a self-contained power supply. Lamps of this type are generally available in synthetic laboratories. The bulbs operated separately. The "short wave" was Lamp B and the "long wave" was Lamp C. Both lamps employ fused silica or UV-transmitting glass envelopes and nonremovable visible-blocking filters. Lamp C also employs a phosphor coating, which absorbs Hg lines below 300 nm and re-emits broadband light from 320 to 400 nm.

*Lamp D.* A UV photochemistry lamp (Unilamp; Midwest Scientific Co.), with a self-contained power supply and a glass envelope that substantially reduces emission below 300 nm. This lamp is equipped with green

and white plastic filters, which were removed prior to use. This is the least expensive of the lamps discussed.

*Monochromator.* Excitation and emission monochromators of a double-grating SPEX Fluorolog 1680 spectrofluorimeter were calibrated. The gratings have 1200 grooves/mm and the monochromators have a 0.22-m focal length with 1.8-nm/mm dispersion and 0.20-nm resolution at 500 nm. The maximum wavelength is 900 nm, which is the limit of the data presented. However, several useful calibration lines exist between 900 and ca. 1200 nm and are listed, although we have not observed them.

*Detector.* Emission spectra were recorded using the standard Hamamatsu R928 PMT in the single-photon counting mode. The detector sensitivity and monochromator transmission characteristics affect the relative observed line intensities but not the spectral positions due to the narrowness of the lines. Spectra presented here are uncorrected for system sensitivity, although this can be done simply by a literature method [10] for the limited spectral range 310–840 nm.

*Scattering Surfaces.* Several scattering surfaces were employed. Spectralon, a polytetrafluoroethylene polymer manufactured by Lab Sphere (North Sutton, NH), is a highly reflective and nearly Lambertian scattering material with an essentially wavelength-independent scattering efficiency over the range 250 to 2400 nm [11]. A  $5 \times 8 \times 0.2$ -cm piece was used as the scattering surface for most spectra. Spectralon is extremely stable but expensive.

Fresh MgO on aluminum was equally effective and very low cost. This surface is prepared by holding a burning Mg ribbon under a small aluminum plate and allowing the MgO "smoke" to coat the aluminum surface thoroughly. MgO provides a nearly Lambertian scattering surface with a highly uniform spectral scattering efficiency [12], but it has a lower stability under UV illumination and shows degradation of UV scattering efficiency over time [12]. A very simple scatterer, consisting of a Kim Wipe folded over a white  $3 \times 5$ -in. note card, was also used.

## PROCEDURE

The lamps were positioned above the scattering surface, which was placed in the spectrofluorimeter sample compartment (Fig. 1). A blackened cardboard baffle with a small slit allowed only scattered light to be detected, which prevented errors due to inconsistent alignment and is a good mimic of sample luminescence. Optical filters

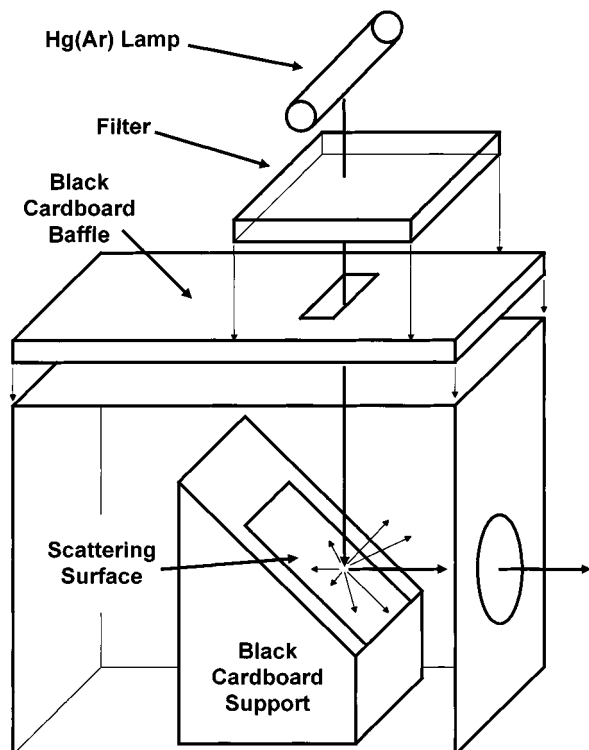


Fig. 1. Exploded schematic of lamp and scattering device assembled in spectrofluorimeter sample compartment.

were employed to discriminate between emission lines of different diffraction orders.

## EMISSION SPECTRA

Initial broad-range spectra were collected at 0.5-nm increments from 200 to 895 nm with a 2-nm resolution. For these measurements, the line spectra were artificially broadened by using asymmetric entrance and exit slit apertures. When both slits are set to narrow apertures, the emission lines are too sharp and may fall between sampling points. If both slits are too wide, the signal intensity is very high and may damage detector components. Also, the resolution and wavelength accuracy are poorer. Asymmetric slit settings produce broad emission signals, with a bandwidth that depends upon the larger slit and a maximum intensity that depends upon the narrow slit. Slit apertures of 0.02 and 0.50 mm (for the entrance and exit slits, respectively) gave effective bandwidths of approximately 1.0 nm. Log-scale emission profiles of the four lamps are shown in Fig. 2. Most monochromators do not allow independent adjustment of the entrance and exit slits, and while the use of asymmet-

ric slits was useful for presenting data over a broad wavelength range, it is neither necessary nor even desirable for calibration. In general, the monochromator calibrations are accurate enough to get reasonable estimates of the line wavelengths. For a grossly out-of-calibration system, the asymmetric slit configuration allows for a very convenient broad spectral scan to locate the lines.

Regions of the spectral lines were scanned again at a high (0.20-nm) resolution, with entrance and exit slits set to 0.02 mm, and data were collected at 0.02-nm increments. These high-resolution scans (Fig. 3) produced bands with slight asymmetry (presumably due to imperfect alignment of dual gratings). Peak positions were assigned as the central wavelength at half-maximum, which typically deviated less than 0.05 nm from the peak maximum. Wavelength measurements among the different lamps agreed to within the instrumental reproducibility of 0.2 nm.

Differences in relative peak intensity of various lamps are easily discernible in Fig. 2. Due to the envelope or filter cutoffs, Lamps C and D show no discernible emission lines below 300 nm and analogously low emission intensities for the corresponding second- and third-order diffraction lines. Both of these sources, however, still produce enough lines for an adequate calibration if better lamps are unavailable. Also, the self-contained visible-blocking filters in Lamps B and C greatly decrease the intensity of first-order lines between 450 and 700 nm. While Lamp B has several moderately intense emission lines in the 500- to 600-nm range, these are due to second-order diffraction of shorter-wavelength lines. The broad emission near 350 nm in Lamp C is due to the lamp phosphors used to maximize emission in this region. Known bands for the four lamps are given in Table I. Although we were able to observe lines only up to 900 nm, longer-wavelength lines are included for use on other instruments; these are included based on their high intensities in the lower orders.

Observed emission intensities depend not only upon lamp characteristics, but also upon instrumental parameters such as detector sensitivity and grating blaze angle. Note, for example, that the second-order diffraction line at 507 nm is more intense than its parent line at 254 nm by nearly an order of magnitude; this is a result of greater grating efficiency in the 500-nm region. For this reason, standardized intensities [7,13] for mercury and argon lines are presented in Table I, although approximate observed intensities may be gleaned from Fig. 2. Subject to the sensitivity characteristics of our detector, the standardized intensities are reasonable guidelines to the intensities to be expected in first order, especially for the closely spaced multiplets. Ar and Hg emission intensities are on different

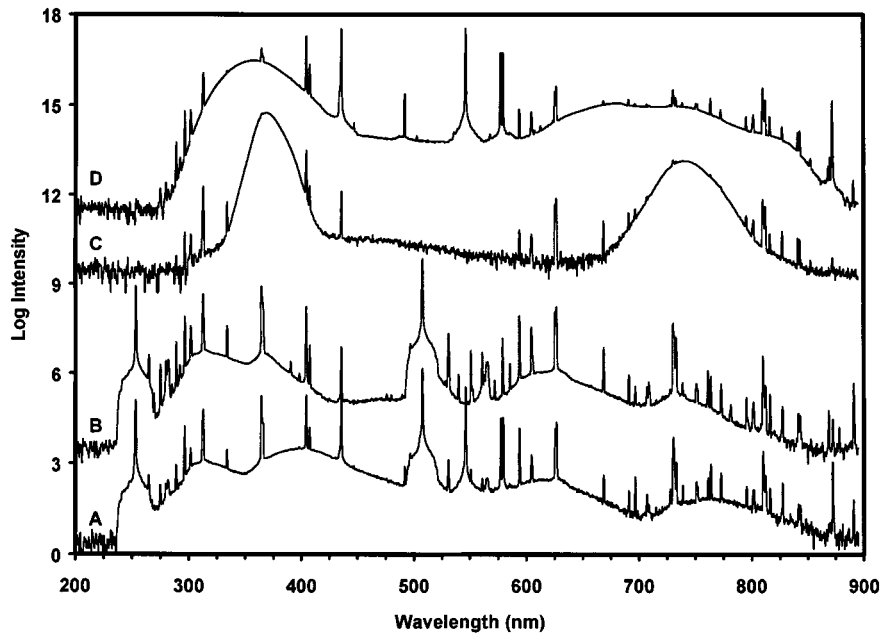


Fig. 2. Log-scale emission profiles of four Hg(Ar) lamps: (a) Lamp A; (b) Lamp B; (c) Lamp C; (d) Lamp D. Spectral baselines are offset for clarity.

relative scales, and intensity comparisons cannot be made between these two emitters.

The resolved spectrum from 809 to 817 nm in Fig. 3a depicts a problem for spectral assignments that is representative of several spectral regions. The existence

of higher order Hg lines and primary Ar lines make these lamps useful over a much broader range than is usually used, but the richer, more complicated emission spectra can make some assignments difficult. Of the four emission lines observed in this region, two are primary Ar

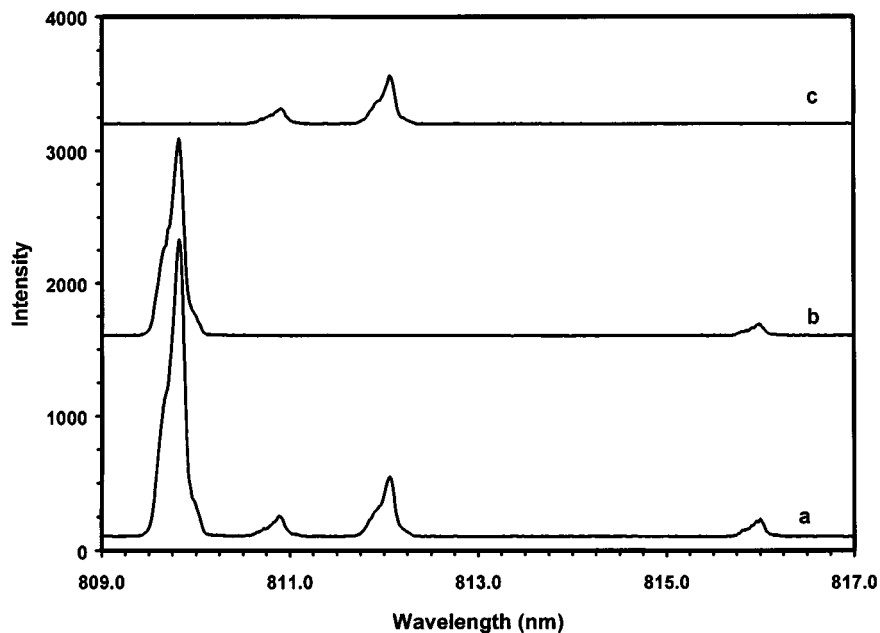


Fig. 3. High-resolution spectra in the 809- to 817-nm region using (a) no filter, (b) a 300- to 500-nm bandpass filter, and (c) a 520-nm cutoff filter. Spectral baselines are offset for clarity.

**Table I.** Known Emission Lines of Mercury and Argon in Low-Pressure Arc Lamps

Relative intensity <sup>a</sup>	Mercury lines (nm)			Argon lines (nm)	
	First order	Second order	Third order	Relative intensity <sup>a</sup>	First order
15,000	253.65	507.30	760.95	10,000	696.54
250	265.20	530.40	795.60	10,000	706.72
400	265.37 <sup>b</sup>	530.74	796.11	1,000	714.70
100	265.51 <sup>b</sup>	531.02	796.53	2,000	727.94
50	269.88	539.76	809.64	10,000	738.40
50	269.94	539.88	809.82	20,000	750.39
80	275.28	550.56	825.84	15,000	751.46
20	275.97	551.94	827.91	25,000	763.51
40	280.35 <sup>b</sup>	560.70	841.05	15,000	772.38
30	280.44 <sup>b</sup>	560.88	841.32	10,000	772.42
	282 <sup>d</sup>	564 <sup>d</sup>	846 <sup>d</sup>	20,000	794.82
50	285.69	571.38	857.07	20,000	800.62
150	289.36	578.72	868.08	25,000	801.48
60	292.54	585.08	877.62	20,000	810.37
1,200	296.73	593.46	890.19	35,000	811.53
300	302.15	604.30	906.45 <sup>c</sup>	10,000	826.45
120	302.35	604.70	907.05 <sup>c</sup>	15,000	840.82
30	302.56	605.12	907.68 <sup>c</sup>	20,000	842.46
50	302.75	605.50	908.25 <sup>c</sup>	15,000	852.14
400	312.57	625.14	937.71 <sup>c</sup>	4,500	866.79
320	313.16 <sup>b</sup>	626.32 <sup>b</sup>	939.48 <sup>c</sup>	35,000	912.30 <sup>c</sup>
320	313.18 <sup>b</sup>	626.36 <sup>b</sup>	939.54 <sup>c</sup>	15,000	922.45 <sup>c</sup>
80	334.15	668.30	1002.45 <sup>c</sup>	1,600	935.42 <sup>c</sup>
2,800	365.02	730.04	1095.06 <sup>c</sup>	25,000	965.78 <sup>c</sup>
300	365.48	730.96	1096.44 <sup>c</sup>	4,500	978.45 <sup>c</sup>
80	366.29 <sup>b</sup>	732.58 <sup>b</sup>	1098.87 <sup>c</sup>	1,600	1,047.00 <sup>c</sup>
240	366.33 <sup>b</sup>	732.66 <sup>b</sup>	1098.99 <sup>c</sup>		
	391 <sup>d</sup>	782 <sup>d</sup>	1173 <sup>c,d</sup>		
	399 <sup>d</sup>	798 <sup>d</sup>	1197 <sup>c,d</sup>		
1,800	404.66	809.32	1213.98 <sup>c</sup>		
150	407.78	815.56			
250	433.92	867.84			
400	434.75	869.50			
4,000	435.83	871.66			
1,100	546.07	1092.14 <sup>c</sup>			
160	567.59	1135.18 <sup>c</sup>			
240	576.96	1153.92 <sup>c</sup>			
100	578.97	1157.94 <sup>c</sup>			
280	579.07	1158.14 <sup>c</sup>			
140	580.38				
250	690.75				
250	708.19				
250	709.19				

<sup>a</sup> Relative intensities are taken from Ref. 13.<sup>b</sup> Not resolved.<sup>c</sup> Not observed in this study; see text.<sup>d</sup> Bands were observed at these positions in more than one lamp but are not assignable to either Hg or Ar.

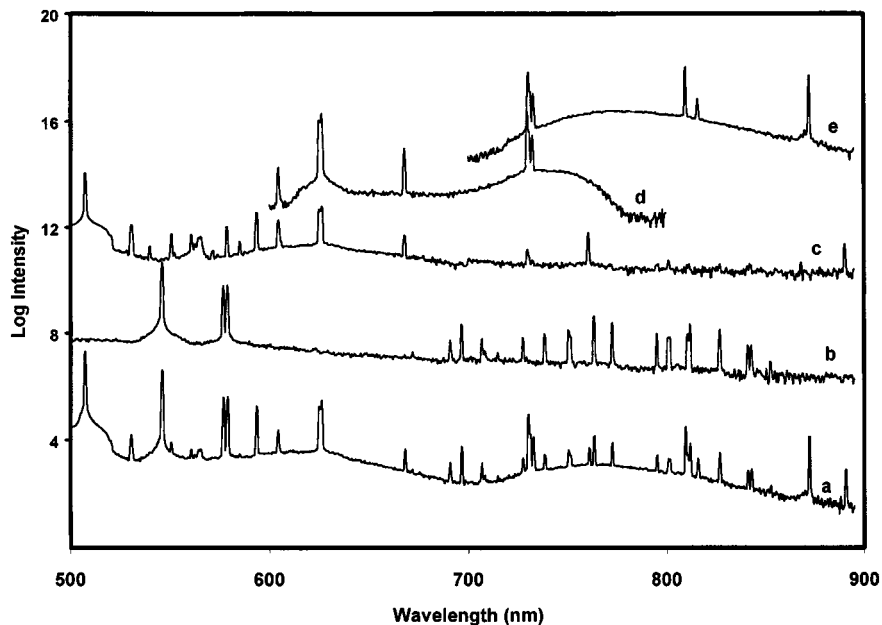
lines and two are second-order diffraction lines due to Hg emission near 400 nm. Because these emission lines

are of different diffraction orders and due to different emitters, assignments cannot be made based on relative intensity. In general, assignments cannot always be made based upon relative spectral position, because it often happens that, due to poorly transmitting envelopes or a low diffraction efficiency, fewer bands are observed in a particular region than are expected. Without knowing which bands are missing, assignments cannot be made with certainty. This problem can be solved by inserting blocking filters that separate bands based on diffraction order. The 520-nm-long wavelength cutoff filter passes primary emission lines in the 800-nm region but blocks the 404- and 408-nm Hg lines in their second order. The reverse is true for the 300- to 500-nm bandpass filter, which passes the 404- and 408-nm Hg lines and blocks the long wavelengths. This separation allows an unambiguous assignment of Hg and Ar lines as shown in Figs. 3 and 4.

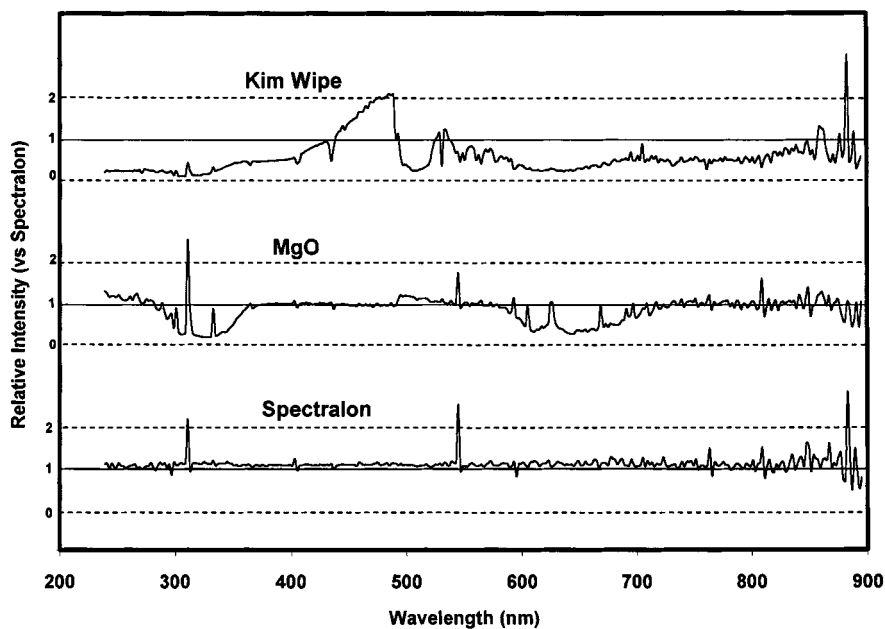
## SCATTERING SURFACES

All three scattering surfaces were adequate. Relative scattering efficiencies for the three surfaces are shown in Fig. 5. For these determinations, the broad-range spectra obtained with MgO and the Kim Wipe were collected and ratioed against the Spectralon spectrum. Spectralon has a scattering efficiency of 0.95 at 250 nm, 0.98 at 300 nm, and approximately 0.99 from 350 nm to > 1 μm [11]. The Spectralon scattering curve is the ratio of two Spectralon spectra and is a measure of experimental reproducibility. As indicated in Fig. 1, the viewing angle and angle of incidence were both approximately 45°. A previous study [11] investigated the geometric dependence of reflectance for this material and found it to be similar to barium sulfate, which is known to be very nearly Lambertian. Although distortions arise from a lack of strong continuum emission from the arc source, relative efficiencies are accurate to approximately ±5% between 250 and 800 nm. Above 500 nm the scattering efficiencies are actually a combination of the scattering efficiencies at the indicated wavelength, λ, and at λ/2 since no effort was made to remove the second-order emission. This effect is demonstrated by the dip in scattering efficiency for MgO at 325 nm, which is repeated at 650 nm. This combined efficiency, however, is appropriate for the use described here.

Freshly prepared MgO is a highly efficient scattering surface and was determined here to have an efficiency near unity, except between 300 and 400 nm, consistent with previous studies [12]. The reflectance near 350 nm is dependent on the film thickness and approaches unity



**Fig. 4.** Broad-range spectra of Lamp A taken with (a) no filter, (b) 500-nm cutoff filter, (c) 250- to 350-nm Bandpass Filter, (d) 320- to 380-nm Bandpass Filter, and (e) 360- to 500-nm bandpass filter. Regions without appreciable intensity were removed to simplify the figure. Spectral baselines are offset for clarity.



**Fig. 5.** Scattering efficiencies of Kim Wipe and magnesium oxide scattering surfaces relative to Spectralon with incident and viewing angles of  $45^\circ$ . The Spectralon scattering efficiency shown here serves as a gauge of experimental reproducibility. Spikes are an artifact of the sharp Hg lines which are superimposed on the broadband spectrum.

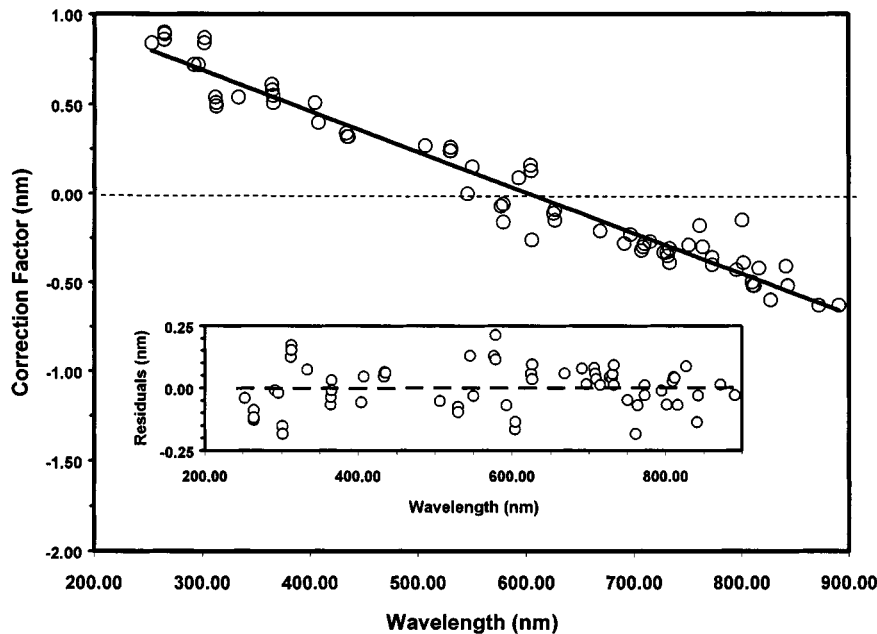


Fig. 6. Plot of wavelength correction factors for emission monochromator calibration. Residuals are displayed in the inset.

in thick layers but is lower here. The scattering efficiency of the Kim Wipe was considerably lower (0.2 to 0.5), with an anomalously high reflectance ( $>1$ ) between 450 and 500 nm, which is due to fluorescence emission of bluing agents added to the paper. While the fluorescence and low reflectance of the Kim Wipe distort the intensities of Hg and Ar lines, their narrow line widths prevent any significant distortion of peak positions. The suboptimal characteristics of the Kim Wipe and MgO reduce signal-to-noise ratios and may make detection and identification of the weak emission lines more difficult; nevertheless, a large number of lines suitable for calibration are still available. This, coupled with their availability and low cost, makes them attractive materials for some users.

## MONOCHROMATOR CALIBRATIONS

Coarse alignment of the emission monochromator was achieved by visual alignment of the bright green 546-nm Hg emission line, as described in the SPEX manual [1]. A rough calibration curve was then created from measurements of approximately five of the strongest emission lines, and the gratings were readjusted iteratively to optimize the calibration near 600 nm. A final, highly accurate calibration was then obtained using the known positions of approximately 60 mercury and argon emission lines. Correction factors, which are the difference between actual and measured wavelengths, were

calculated for each point, and fit by least squares to a linear function of wavelength, as shown in Fig. 6. Corrected wavelengths are obtained by adding the correction factor to the measured wavelength. The accuracy of the calibration is shown by the residuals ( $SD = 0.10$  nm) in the inset to Fig. 6, which are the differences between actual wavelengths and those calculated using the linearized correction factors.

The excitation monochromator was calibrated using the calibrated emission monochromator by the standard method [2]. The emission monochromator was set at a fixed wavelength with narrow slits. The excitation monochromator, also with narrow slits, was scanned over this wavelength while scattering the excitation beam into the emission monochromator with a scattering surface positioned in the sample compartment at the cuvette position. This scatterer reflects a monochromatized portion of the broadband Xe excitation source into the emission monochromator. The detector registers a spike when the two monochromators pass light of exactly the same wavelength. This process was repeated at a number of wavelengths to generate an excitation monochromator calibration curve. Results were comparable to those for the emission monochromator.

## CONCLUSIONS

A simple procedure for calibrating emission monochromators is described using emission lines from a mer-

cury vapor lamp and is applied to a SPEX 1680 Fluorolog spectrofluorimeter. Previous calibration methods using only first-order Hg lines were severely limited in both the number (typically  $\sim 10$ ) and the range (250–600 nm) of usable emission lines. The present method uses both higher-order Hg lines and Ar lines, providing more than 60 emission lines extending from 250 nm well into the near-infrared. Four different lamps were found to be satisfactory, although variations in lamp materials and filters greatly reduce the number and intensity of the available emission lines for some lamps. The excitation monochromator was calibrated using the emission monochromator.

Of the three scattering surfaces used (Spectralon, MgO, and a Kim Wipe), Spectralon has the highest and most constant scattering efficiency but is the most expensive. The other two surfaces may also be used successfully.

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