you-try-it-04.xlsx
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For use with:
Brian M. Tissue, *Basics of Analytical Chemistry and Chemical Equilibria,* (John Wiley: New York, 2013).
http://www.achem.org

Worksheets in this file

notes	This page with background information.
4.A conversions	Convert measured wavelength to vacuum wavelength and energy.
4.B absorbance	Calculate transmittance (T), percent-transmittance (%-T), and absorbance.
4.C Beers-law	Use the Beer-Lambert law to find concentration from absorbance.
4.D fluorescence	Determine an unknown using a fluorescence calibration curve.

Background
Refer to Chapter 4 in the text for equations and explanations.
Each worksheet has instructions in the shaded box.
For answers and step-by-step help see you-try-it-04guide.pdf.

You-Try-It 4.A **Wavelength to Energy Conversion**

Tables 4.A.1 and 4.A.2 list spectroscopic transitions of neutral Na and K.
The first column gives a relative intensity for each transition (not needed for our calculation).
The second column gives the wavelength as measured in air (Å=10^{-10} m).
Columns H and I give accepted energy levels that appear to match the measured wavelengths.
The energy levels are the best fit from multiple measurements and converted to vacuum energies.
Convert the wavelengths measured in air to vacuum wavelengths to confirm that they correspond to transitions between the listed levels. The refractive index of air is given in cell G19.

1. Convert the wavelengths measured in air to wavelength in vacuum.
2. Convert the vacuum wavelength to energy in cm^{-1}.

conversions/constants:

Å/m	Å/cm	c_{vac} (m/s)	n (air)	c_{air} (m/s)
1.0E+10	1.0E+08	299792458	1.000293	299704644.5

Table 4.A.1
Persistent Lines of Neutral Sodium (Na I)

Intensity	λ (air) (Å)	Frequency (s^{-1})	λ (vac) (Å)	E (meas) (cm^{-1})	E (theor) (cm^{-1})	Energy Levels (cm^{-1}) lower	upper
5	2852.810					0.000	35042.850
2	2853.010					0.000	35040.380
15	3302.370					0.000	30272.580
8	3302.980					0.000	30266.990
1000	5889.950					0.000	16973.368
500	5895.924					0.000	16956.172

Table 4.A.2
Persistent Lines of Neutral Potassium (K I)

Intensity	λ (air) (Å)	Frequency (s^{-1})	λ (vac) (Å)	E (meas) (cm^{-1})	E (theor) (cm^{-1})	Energy Levels (cm^{-1}) lower	upper
700	4044.140					0.000	24720.139
700	4047.210					0.000	24701.382
1000	7664.899					0.000	13042.876
1000	7698.965					0.000	12985.170
700	11690.210					12985.170	21536.988
600	11769.620					13042.876	21536.988
700	11772.830					13042.876	21534.680

Data from:
J.E. Sansonetti, W.C. Martin, and S.L. Young (2005),
Handbook of Basic Atomic Spectroscopic Data (version 1.1.2).
[Online] Available: http://physics.nist.gov/Handbook [2009, June 3].
National Institute of Standards and Technology, Gaithersburg, MD

You-Try-It 4.B Absorbance, Transmittance, and Percent Transmittance

Table 4.B.1 gives data collected with a homemade colorimeter to measure Cu^{2+} in pool water.
(Copper is sometimes used as an algicide, but at high concentrations it can turn blond hair greenish.)
The colorimeter consists of a blue LED source and a light sensor mounted in a box.
An opening in the box allows positioning a test tube in the light path.
Given that the optical arrangement does not change, the lux measurements are comparable to light power.
The added reagent creates a colored complex that absorbs at the wavelength of the LED.
Reagent addition (5 drops) does not cause a measurable change in the solution volume.
A copper concentration of 1.0 ppm is known to produce an absorbance of 0.727 using this procedure.

1. Calculate *T* , %-*T* , and *A* for the light measurements.
2. Calculate the copper concentration in the pool water given the calibration data above.

Table 4.B.2 lists repetitive measurements of the pool water (the blank).

3. Use the data in Table 4.B.2 to predict the LOD and LOQ for this method.

Table 4.B.1

sample	measurement (lux)
nothing in the light path	450
test tube of pool water	399
pool water + reagent	211

Table 4.B.2 (pool water only)

trial	measurement (lux)
1	399
2	389
3	406
4	394

You-Try-It 4.C **Beer-Lambert Law**

The data in Table 4.C.1 are absorbance vs wavelength for a standard riboflavin solution.
The data in Table 4.C.2 are absorbance vs wavelength for an unknown solution.
Both data sets were made with identical instrument settings.

1. Insert a scatter chart to plot both data sets and adjust axes and formatting.
2. Determine the maximum absorbance values for the peaks near 260 and 450 nm.
   Calculate the molar absorptivity for each peak wavelength.
3. Determine the concentration of the unknown solution.

Table 4.C.1 Standard Solution

analyte	riboflavin
solvent	ethanol
b (cm)	1.00
c_{std} (M)	2.00E-05

λ (nm)	***A***
219	0.8475
220	0.8662
221	0.8623
222	0.8320
223	0.7869
224	0.7367

Table 4.C.2 Unknown Solution

analyte	riboflavin
solvent	ethanol
b (cm)	1.00
c_{unk}	?

λ (nm)	***A***
219	0.2418
220	0.2348
221	0.2269
222	0.2227
223	0.2108
224	0.1974

225	0.6882	225	0.1845
226	0.6418	226	0.1730
227	0.5979	227	0.1571
228	0.5591	228	0.1460
229	0.5257	229	0.1353
230	0.4985	230	0.1261
231	0.4768	231	0.1316
232	0.4591	232	0.1234
233	0.4450	233	0.1200
234	0.4346	234	0.1279
235	0.4280	235	0.1111
236	0.4255	236	0.1159
237	0.4263	237	0.1127
238	0.4304	238	0.1185
239	0.4376	239	0.1234
240	0.4468	240	0.1269
241	0.4574	241	0.1309
242	0.4691	242	0.1215
243	0.4811	243	0.1248
244	0.4934	244	0.1349
245	0.5070	245	0.1303
246	0.5209	246	0.1423
247	0.5349	247	0.1500
248	0.5478	248	0.1553
249	0.5597	249	0.1592
250	0.5703	250	0.1513
251	0.5799	251	0.1540
252	0.5883	252	0.1543
253	0.5977	253	0.1605
254	0.6083	254	0.1643
255	0.6209	255	0.1720
256	0.6355	256	0.1758
257	0.6514	257	0.1839
258	0.6673	258	0.1853
259	0.6819	259	0.1806
260	0.6940	260	0.1903
261	0.7030	261	0.1933
262	0.7086	262	0.1846
263	0.7095	263	0.1916
264	0.7042	264	0.1882
265	0.6920	265	0.1760
266	0.6734	266	0.1806
267	0.6509	267	0.1806
268	0.6269	268	0.1667
269	0.6028	269	0.1645
270	0.5785	270	0.1548
271	0.5537	271	0.1578
272	0.5266	272	0.1408
273	0.4969	273	0.1326
274	0.4660	274	0.1252
275	0.4344	275	0.1244
276	0.4031	276	0.1173
277	0.3727	277	0.1084

278	0.3437	278	0.1033
279	0.3154	279	0.0866
280	0.2876	280	0.0739
281	0.2607	281	0.0746
282	0.2346	282	0.0640
283	0.2098	283	0.0552
284	0.1853	284	0.0580
285	0.1618	285	0.0480
286	0.1401	286	0.0392
287	0.1205	287	0.0366
288	0.1026	288	0.0263
289	0.0873	289	0.0225
290	0.0740	290	0.0202
291	0.0628	291	0.0238
292	0.0533	292	0.0149
293	0.0465	293	0.0189
294	0.0413	294	0.0306
295	0.0374	295	0.0119
296	0.0348	296	0.0090
297	0.0336	297	0.0077
298	0.0331	298	0.0147
299	0.0336	299	0.0147
300	0.0350	300	0.0164
301	0.0364	301	0.0158
302	0.0385	302	0.0193
303	0.0410	303	0.0126
304	0.0439	304	0.0186
305	0.0470	305	0.0212
306	0.0503	306	0.0130
307	0.0536	307	0.0207
308	0.0571	308	0.0112
309	0.0607	309	0.0235
310	0.0645	310	0.0200
311	0.0682	311	0.0195
312	0.0719	312	0.0218
313	0.0757	313	0.0244
314	0.0796	314	0.0224
315	0.0837	315	0.0286
316	0.0877	316	0.0343
317	0.0920	317	0.0384
318	0.0960	318	0.0396
319	0.1005	319	0.0297
320	0.1045	320	0.0264
321	0.1089	321	0.0242
322	0.1134	322	0.0394
323	0.1177	323	0.0294
324	0.1218	324	0.0331
325	0.1260	325	0.0332
326	0.1298	326	0.0372
327	0.1336	327	0.0376
328	0.1374	328	0.0382
329	0.1405	329	0.0389
330	0.1438	330	0.0328

331	0.1467	331	0.0464
332	0.1495	332	0.0402
333	0.1519	333	0.0367
334	0.1543	334	0.0392
335	0.1564	335	0.0450
336	0.1584	336	0.0451
337	0.1602	337	0.0505
338	0.1616	338	0.0414
339	0.1631	339	0.0488
340	0.1644	340	0.0465
341	0.1654	341	0.0502
342	0.1664	342	0.0494
343	0.1671	343	0.0457
344	0.1675	344	0.0486
345	0.1679	345	0.0416
346	0.1680	346	0.0435
347	0.1679	347	0.0558
348	0.1678	348	0.0434
349	0.1671	349	0.0383
350	0.1662	350	0.0443
351	0.1653	351	0.0411
352	0.1646	352	0.0521
353	0.1635	353	0.0418
354	0.1623	354	0.0454
355	0.1612	355	0.0527
356	0.1602	356	0.0421
357	0.1591	357	0.0532
358	0.1584	358	0.0482
359	0.1584	359	0.0420
360	0.1585	360	0.0480
361	0.1583	361	0.0342
362	0.1587	362	0.0427
363	0.1597	363	0.0438
364	0.1604	364	0.0425
365	0.1603	365	0.0466
366	0.1583	366	0.0462
367	0.1553	367	0.0433
368	0.1525	368	0.0434
369	0.1515	369	0.0433
370	0.1516	370	0.0433
371	0.1513	371	0.0496
372	0.1504	372	0.0464
373	0.1491	373	0.0376
374	0.1478	374	0.0401
375	0.1459	375	0.0363
376	0.1440	376	0.0439
377	0.1417	377	0.0421
378	0.1400	378	0.0454
379	0.1385	379	0.0358
380	0.1368	380	0.0416
381	0.1352	381	0.0303
382	0.1333	382	0.0482
383	0.1317	383	0.0459

384	0.1303	384	0.0282
385	0.1294	385	0.0363
386	0.1284	386	0.0411
387	0.1276	387	0.0430
388	0.1266	388	0.0398
389	0.1260	389	0.0272
390	0.1251	390	0.0337
391	0.1248	391	0.0361
392	0.1246	392	0.0405
393	0.1242	393	0.0355
394	0.1237	394	0.0354
395	0.1233	395	0.0465
396	0.1234	396	0.0323
397	0.1236	397	0.0388
398	0.1236	398	0.0451
399	0.1239	399	0.0438
400	0.1236	400	0.0343
401	0.1239	401	0.0321
402	0.1243	402	0.0356
403	0.1240	403	0.0416
404	0.1235	404	0.0372
405	0.1244	405	0.0279
406	0.1240	406	0.0394
407	0.1238	407	0.0297
408	0.1237	408	0.0396
409	0.1237	409	0.0371
410	0.1233	410	0.0306
411	0.1225	411	0.0324
412	0.1221	412	0.0357
413	0.1215	413	0.0387
414	0.1204	414	0.0229
415	0.1196	415	0.0309
416	0.1191	416	0.0294
417	0.1189	417	0.0362
418	0.1187	418	0.0358
419	0.1183	419	0.0308
420	0.1184	420	0.0362
421	0.1189	421	0.0315
422	0.1194	422	0.0301
423	0.1197	423	0.0341
424	0.1204	424	0.0428
425	0.1213	425	0.0322
426	0.1225	426	0.0328
427	0.1241	427	0.0349
428	0.1255	428	0.0229
429	0.1275	429	0.0372
430	0.1297	430	0.0367
431	0.1310	431	0.0404
432	0.1324	432	0.0378
433	0.1348	433	0.0368
434	0.1378	434	0.0360
435	0.1393	435	0.0385
436	0.1416	436	0.0284

437	0.1438	437	0.0488
438	0.1459	438	0.0430
439	0.1478	439	0.0422
440	0.1489	440	0.0407
441	0.1508	441	0.0437
442	0.1523	442	0.0492
443	0.1528	443	0.0368
444	0.1533	444	0.0369
445	0.1539	445	0.0433
446	0.1542	446	0.0479
447	0.1544	447	0.0349
448	0.1541	448	0.0421
449	0.1534	449	0.0463
450	0.1526	450	0.0437
451	0.1516	451	0.0417
452	0.1507	452	0.0513
453	0.1492	453	0.0291
454	0.1482	454	0.0452
455	0.1466	455	0.0383
456	0.1453	456	0.0297
457	0.1439	457	0.0497
458	0.1425	458	0.0409
459	0.1412	459	0.0457
460	0.1394	460	0.0387
461	0.1384	461	0.0402
462	0.1373	462	0.0417
463	0.1365	463	0.0347
464	0.1354	464	0.0417
465	0.1340	465	0.0355
466	0.1327	466	0.0438
467	0.1314	467	0.0381
468	0.1308	468	0.0460
469	0.1294	469	0.0429
470	0.1282	470	0.0484
471	0.1266	471	0.0344
472	0.1249	472	0.0391
473	0.1227	473	0.0256
474	0.1196	474	0.0369
475	0.1169	475	0.0409
476	0.1136	476	0.0375
477	0.1096	477	0.0461
478	0.1064	478	0.0260
479	0.1018	479	0.0251
480	0.0974	480	0.0282
481	0.0927	481	0.0278
482	0.0875	482	0.0180
483	0.0828	483	0.0160
484	0.0775	484	0.0274
485	0.0718	485	0.0354
486	0.0648	486	0.0231
487	0.0587	487	0.0218
488	0.0545	488	0.0182
489	0.0508	489	0.0066

490	0.0467	490	0.0183
491	0.0418	491	0.0159
492	0.0377	492	0.0134
493	0.0332	493	0.0168
494	0.0296	494	0.0128
495	0.0263	495	0.0107
496	0.0228	496	0.0115
497	0.0197	497	0.0071
498	0.0169	498	0.0050
499	0.0148	499	0.0082
500	0.0124	500	0.0086
501	0.0103	501	0.0048
502	0.0088	502	0.0032
503	0.0073	503	0.0032
504	0.0060	504	0.0079
505	0.0053	505	0.0045
506	0.0043	506	-0.0017
507	0.0034	507	0.0010
508	0.0030	508	0.0017
509	0.0029	509	0.0056
510	0.0021	510	0.0006
511	0.0019	511	-0.0002
512	0.0016	512	0.0085
513	0.0014	513	-0.0043
514	0.0014	514	0.0044
515	0.0017	515	-0.0017
516	0.0017	516	0.0028
517	0.0015	517	0.0046
518	0.0016	518	0.0017
519	0.0019	519	0.0054
520	0.0019	520	0.0093
521	0.0022	521	-0.0041
522	0.0021	522	0.0031
523	0.0024	523	0.0035
524	0.0026	524	0.0051
525	0.0026	525	-0.0047
526	0.0023	526	0.0079
527	0.0027	527	0.0093
528	0.0026	528	0.0063
529	0.0028	529	0.0005
530	0.0030	530	0.0035
531	0.0031	531	0.0008
532	0.0029	532	0.0049
533	0.0033	533	0.0075
534	0.0030	534	0.0051
535	0.0031	535	-0.0004
536	0.0030	536	-0.0076
537	0.0033	537	0.0062
538	0.0036	538	0.0065
539	0.0035	539	-0.0044
540	0.0035	540	0.0037
541	0.0035	541	0.0018
542	0.0035	542	0.0088

543	0.0035	543	0.0056
544	0.0036	544	0.0056
545	0.0039	545	0.0008
546	0.0034	546	0.0038
547	0.0038	547	-0.0052
548	0.0037	548	0.0030
549	0.0036	549	0.0072
550	0.0037	550	0.0009
551	0.0041	551	0.0030
552	0.0040	552	0.0082
553	0.0040	553	0.0063
554	0.0041	554	-0.0007
555	0.0042	555	0.0022
556	0.0044	556	-0.0067
557	0.0040	557	0.0009
558	0.0040	558	0.0127
559	0.0043	559	0.0094
560	0.0046	560	0.0061
561	0.0045	561	0.0011
562	0.0041	562	-0.0014
563	0.0041	563	0.0031
564	0.0047	564	0.0004
565	0.0045	565	0.0042
566	0.0043	566	-0.0025
567	0.0042	567	-0.0009
568	0.0041	568	0.0062
569	0.0039	569	-0.0018
570	0.0039	570	0.0081
571	0.0033	571	0.0006
572	0.0029	572	-0.0007
573	0.0028	573	0.0033
574	0.0032	574	0.0083
575	0.0029	575	0.0041
576	0.0027	576	0.0045
577	0.0026	577	-0.0014
578	0.0030	578	-0.0068
579	0.0020	579	-0.0010
580	0.0026	580	0.0011
581	0.0026	581	0.0083
582	0.0029	582	0.0028
583	0.0015	583	0.0005
584	0.0024	584	0.0094
585	0.0034	585	0.0041
586	0.0027	586	-0.0011
587	0.0027	587	0.0034
588	0.0030	588	-0.0001
589	0.0031	589	-0.0001
590	0.0036	590	0.0014
591	0.0040	591	-0.0026
592	0.0039	592	-0.0019
593	0.0037	593	-0.0086
594	0.0034	594	0.0138
595	0.0033	595	0.0004

596	0.0038	596	0.0004
597	0.0039	597	0.0091
598	0.0039	598	0.0034
599	0.0039	599	0.0023
600	0.0036	600	0.0056
601	0.0035	601	0.0036
602	0.0035	602	0.0063
603	0.0037	603	0.0019
604	0.0038	604	0.0043
605	0.0037	605	0.0174
606	0.0037	606	0.0059
607	0.0037	607	0.0099
608	0.0038	608	-0.0037
609	0.0039	609	0.0076
610	0.0040	610	0.0005
611	0.0039	611	0.0035
612	0.0041	612	0.0097
613	0.0037	613	0.0077
614	0.0038	614	0.0005
615	0.0038	615	0.0008
616	0.0039	616	0.0013
617	0.0041	617	0.0008
618	0.0043	618	0.0067
619	0.0046	619	0.0026
620	0.0046	620	0.0132
621	0.0046	621	-0.0025
622	0.0043	622	0.0045
623	0.0040	623	0.0107
624	0.0036	624	0.0016
625	0.0036	625	0.0130
626	0.0042	626	0.0047
627	0.0044	627	0.0001
628	0.0048	628	0.0041
629	0.0048	629	0.0038
630	0.0046	630	0.0097
631	0.0042	631	0.0074
632	0.0035	632	0.0028
633	0.0036	633	0.0032
634	0.0036	634	0.0049
635	0.0037	635	0.0124
636	0.0038	636	0.0082
637	0.0042	637	0.0018
638	0.0046	638	0.0002
639	0.0050	639	0.0028
640	0.0049	640	-0.0009
641	0.0052	641	-0.0006
642	0.0047	642	0.0009
643	0.0046	643	0.0184
644	0.0048	644	-0.0053
645	0.0046	645	-0.0025
646	0.0048	646	0.0047
647	0.0046	647	-0.0008
648	0.0045	648	0.0115

649	0.0042	649	0.0051
650	0.0042	650	0.0112
651	0.0044	651	0.0095
652	0.0041	652	-0.0042
653	0.0042	653	0.0053
654	0.0048	654	0.0063
655	0.0010	655	0.0022
656	-0.0025	656	-0.0024
657	-0.0014	657	0.0059
658	0.0011	658	0.0022
659	0.0036	659	-0.0035
660	0.0040	660	-0.0070
661	0.0039	661	0.0058
662	0.0038	662	0.0082
663	0.0035	663	-0.0103
664	0.0037	664	-0.0004
665	0.0037	665	0.0073
666	0.0035	666	0.0047
667	0.0034	667	-0.0064
668	0.0032	668	0.0024
669	0.0032	669	0.0040
670	0.0029	670	0.0066
671	0.0028	671	0.0047
672	0.0023	672	0.0044
673	0.0024	673	0.0028
674	0.0025	674	-0.0061
675	0.0025	675	-0.0023
676	0.0022	676	0.0087
677	0.0023	677	0.0070
678	0.0019	678	0.0039
679	0.0019	679	0.0039
680	0.0018	680	0.0000
681	0.0019	681	-0.0002
682	0.0017	682	0.0092
683	0.0018	683	-0.0078
684	0.0014	684	0.0029
685	0.0012	685	-0.0050
686	0.0012	686	-0.0019
687	0.0013	687	0.0039
688	0.0011	688	-0.0009
689	0.0012	689	-0.0092
690	0.0009	690	0.0137
691	0.0008	691	0.0079
692	0.0006	692	0.0099
693	0.0005	693	-0.0021
694	0.0008	694	-0.0010
695	0.0006	695	0.0037
696	0.0004	696	0.0072
697	0.0000	697	-0.0027
698	0.0000	698	0.0051
699	0.0000	699	0.0064
700	-0.0001	700	0.0094

data from: H. Du, R.-C. A. Fuh, J. Li, A. Corkan, J. S. Lindsey,
PhotochemCAD: A computer-aided design and research tool in photochemistry,
Photochemistry and Photobiology,68, 141-142, (1998)
DOI: 10.1111/j.1751-1097.1998.tb02480.x
Program and databases available at:
http://omlc.ogi.edu/spectra/PhotochemCAD/html/du98.html

You-Try-It 4.D Fluorescence Data

Table 4.D.1 provides multiple measurements of riboflavin fluorescence for standards and an unknown.
All samples were measured with identical instrument settings.
The unknown solution was prepared by the following procedure:

Dissolve 1.0 g dry sample (infant formula) in 10 mL of 0.1 M HCl.
Autoclave 30 min at 122 C, cool, and neutralize to pH 6 with NaOH solution.
Adjust to pH 4.5 with dilute HCl and filter.
Dilute solution to 50.0 mL and measure fluorescence at 565 nm.

1. Determine the mean and standard deviation of all repetitive measurements.
   Create a calibration curve of the fluorescence vs concentration.
2. Determine the riboflavin concentration in the unknown solution.
3. Determine the riboflavin concentration and uncertainty in the unknown dry sample.
   The sample size of the dry powder was 1.080 g.

Table 4.D.1 Fluorescence Measurements

conc (ppm)	1	2	3	4
0.00	0.01	0.00	0.00	
0.01	0.11	0.11	0.11	
0.02	0.24	0.24	0.24	
0.04	0.50	0.50	0.50	
0.08	0.96	0.97	0.93	
0.10	1.25	1.24	1.24	
unknown	0.75	0.74	0.77	0.77

average	std dev

Procedure adapted from:
AOAC Official Method 970.65, Riboflavin in Foods and Vitamin Preparations.