APV
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Technical Bulletin

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Understanding the
Gaulin EQA™ Analyzer

The turbidity of a diluted sample of an emulsion such as butterfat in water is a measure of how much light is scattered by the butterfat globules. The amount of scattered light is a function of the average particle size of the butterfat globules, the concentration or amount of fat in the sample and the difference in the refractive index between water and butterfat. A spectrophotometer with optical modifications can be used to measure the degree of light scattering in an emulsion when various parameters of the emulsion are known.

The concentration of fat is readily determined by various methods. The refractive indices of distilled water and butterfat are known. With this information the average particle size can be determined with the spectrophotometer, using seven different wavelengths of light (from 400 - 1000 nm) by measuring the transmitted light and not the scattered light. These seven wavelengths are such that they will detect all particle sizes from about two-tenths of a micrometer to the maximum diameter expected in the emulsion. By applying these seven readings to a complicated formula the average particle size can be determined. The EQA instrument is a simplified form of a standard spectrophotometer and is capable of determining the mean droplet diameter for a milk emulsion.

The mean droplet diameter of the milk emulsion is a measure of its rate of creaming. "Creaming" (fat rise or fat ring) is caused by the amount of oil in the emulsion which floats to the top. Particles of 0.5 micrometers or less in size do not readily separate and float to the top. Particles measuring one micrometer and larger rise more readily and also contain the greatest amount of oil.

In an oil-in-water emulsion such as homogenized milk, the oil phase consists of fat globules. The volume of a globule is proportional to the diameter cubed. For example, it requires 1,000 particles (N_2) 0.5 micrometer (µm) in diameter (d_2) to equal the volume of one (N_1), 5 micrometer particle (d_1).

$$N_1V_1 = N_1V_1 \text{ where } V = 1/6\,\pi d^3$$

$$N_2 = N_1 \frac{V_1}{V_2} = 1 * \frac{1/6\,\pi(5)^3}{1/6\,\pi(0.5)^3} = 1000$$

In an emulsion containing six, 5 µm particles and 6000 particles of 0.5 µm in diameter, the oil in the emulsion would be equally divided between the large and small particles. If the six, 5 µm particles floated, then 50% of the oil would be on top. If four of the six, 5 µm particles are reduced to 0.5 µm particles, there would then be (4 x 1000) + 6000 = 10,000 particles of 0.5 µm which would contain 83-1/3% of the oil in the emulsion. The remaining two, 5 µm particles will contain 16-2/3% rather than 50% of the oil. Therefore, reducing the number of large globules decreases the rate of creaming in the emulsion.

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By using 1 wavelength of light rather than 7, the EQA can detect particle sizes from about 0.5 to 3 µm. Having this information permits use of an "Absorbance Index" chart provided with the EQA. Absorbance, a photometric term, is equal to the negative logarithm of light transmittance.

By noting the absorbance index reading, determined when a properly prepared sample is inserted into the EQA, and knowing the fat content, an average fat-globule diameter can be read directly from the chart. The chart was developed by analyzing data from a spectrophotometer for many milk emulsions and transforming the information by algorithms to the EQA instrument format.

To test dairy products with fat content beyond the range shown on the chart, dilute the sample sufficiently to bring the estimated fat content within the chart range, test the diluted sample for fat content and insert the prepared sample in the EQA.

The EQA can be used on certain other oil-in-water emulsions, providing the emulsion is dispersible in water, the expected maximum diameter of the oil globule does not exceed 3 µm and the sample does not contain insoluble solids, which will interfere with the light scattering. By measuring a series of acceptable product samples, a range of absorbance index numbers can be generated. Future samples within this range would indicate an emulsion of similar quality to the acceptable product samples.

Comparison of the EQA Reading to Creaming Rate

The mean diameter determined by the EQA is called d_{43}. The d_{43} is the moment/volume mean diameter and is the mean diameter of the volume-droplet-size distribution. It is a measure of how the total volume of oil is distributed among the different-sized droplets. In many cases, the value of d_{43} is the most convenient average diameter to use for characterizing emulsion quality, because it takes into account the volume/surface mean diameter and also the droplet-size distribution.

The question that arises regarding the use of d_{43} pertains to how it relates to the creaming rate of the homogenized milk. To answer this question two different techniques that measure degree of creaming were tested and compared to the turbidity results.

The first method is the U. S. Public Health Service Index for homogenizing efficiency, also known as the top and bottom butterfat determination. At one time this method was used as a standard for homogenization but is not used today. First, samples were analyzed using spectroturbidity and then were tested for top and bottom fat content after storage for 48 hours at 40° F. The top and bottom test was performed by removing 100 mL of milk from the top of a one-quart sample that had been stored for 48 hours. The fat content of this top portion was determined by a modified Babcock test. The fat content of the remaining bottom portion was also measured. An index was then determined by subtracting the fat percent of the bottom portion from the fat percent of the top portion and then by dividing this remainder by the percentage of the top portion and multiplying by 100.

The second method is the N.I.Z.O. pipet method of homogenizing efficiency. N.I.Z.O stands for the Netherlands Institute for Dairy Research. The pipet holds about 25 mL of milk. The pipet is filled with milk to an upper line marking on the tube. The tube is sealed and then spun in a heated centrifuge (40°C) for thirty minutes. After centrifuging, the milk is drained out of the pipet until the upper level reaches a bottom level line on the tube. This leaves about 5 mL of milk in the pipet (about the top 20% of the sample) which is discarded. The bottom portion of the sample is analyzed for percent butterfat, and this percentage is divided by the butterfat percentage for the original milk sample and multiplied by 100. The larger the number is, the better the homogenization. Homogenized milk normally should have a value between 70 and 90 %.

The attached graph shows the correlation between the mean diameter (d_{43}) measured by the EQA and these two creaming tests. It is obvious that the EQA is a good measure of the rate of creaming of a milk sample.

ABSORBANCE INDEX CHART I

Percentage of Butterfat Sample

Average Fat Globule Diameter

	2.60	2.70	2.80	2.90	3.00	3.10	3.20	3.30	3.40	3.50	3.60	3.70	3.80	3.90	4.00	4.10	4.20
0.400	.077	.080	.083	.086	.089	.092	.095	.098	.101	.104	.107	.110	.113	.116	.118	.121	.124
0.425	.085	.088	.092	.095	.098	.102	.105	.108	.111	.115	.118	.121	.124	.128	.131	.134	.138
0.450	.093	.097	.100	.104	.108	.111	.115	.118	.122	.126	.129	.133	.136	.140	.144	.147	.151
0.475	.101	.105	.109	.113	.117	.121	.125	.129	.133	.137	.140	.144	.148	.152	.156	.160	.164
0.500	.110	.114	.118	.122	.126	.131	.135	.139	.143	.147	.152	.156	.160	.164	.169	.173	.177
0.525	.118	.122	.127	.131	.136	.140	.145	.149	.154	.158	.163	.167	.172	.177	.181	.186	.190
0.550	.126	.131	.135	.140	.145	.150	.155	.160	.165	.169	.174	.179	.184	.180	.194	.198	.203
0.575	.134	.139	.144	.149	.155	.160	.165	.170	.175	.180	.185	.191	.196	.201	.206	.211	.216
0.600	.142	.148	.153	.158	.164	.169	.175	.180	.186	.191	.197	.202	.208	.213	.219	.224	.230
0.625	.150	.156	.162	.168	.173	.179	.185	.191	.196	.202	.208	.214	.220	.225	.231	.237	.243
0.650	.158	.164	.171	.177	.183	.189	.195	.201	.207	.213	.219	.225	.231	.238	.244	.250	.256
0.675	.166	.173	.179	.186	.192	.198	.205	.211	.218	.224	.231	.237	.243	.250	.256	.263	.269
0.700	.179	.186	.192	.199	.206	.213	.220	.227	.234	.241	.247	.254	.261	.268	.275	.282	.289
0.725	.185	.192	.199	.206	.213	.221	.228	.235	.242	.249	.256	.263	.270	.277	.285	.292	.299
0.750	.191	.199	.206	.213	.221	.228	.235	.243	.250	.257	.265	.272	.280	.287	.294	.302	.309
0.775	.198	.205	.213	.220	.228	.236	.243	.251	.258	.266	.274	.281	.289	.296	.304	.312	.319
0.800	.204	.212	.220	.227	.235	.243	.251	.259	.267	.274	.282	.290	.298	.306	.314	.322	.329
0.825	.210	.218	.226	.234	.243	.251	.259	.267	.275	.283	.291	.299	.307	.315	.323	.331	.340
0.850	.217	.225	.233	.242	.250	.258	.266	.275	.283	.291	.300	.308	.316	.325	.333	.341	.350
0.875	.223	.231	.240	.249	.257	.266	.274	.283	.291	.300	.309	.317	.326	.334	.343	.351	.360
0.900	.229	.238	.247	.256	.264	.273	.282	.291	.300	.308	.317	.326	.335	.344	.353	.361	.370
0.925	.235	.245	.254	.263	.272	.281	.290	.299	.308	.317	.326	.335	.344	.353	.362	.371	.380
0.950	.242	.251	.260	.270	.279	.288	.298	.307	.316	.325	.335	.344	.353	.363	.372	.381	.391
0.975	.248	.258	.267	.277	.286	.296	.305	.315	.324	.334	.344	.353	.363	.372	.382	.391	.401
1.000	.254	.264	.274	.284	.294	.303	.313	.323	.333	.342	.352	.362	.372	.382	.391	.401	.411
1.025	.261	.271	.281	.291	.301	.311	.321	.331	.341	.351	.361	.371	.381	.391	.401	.411	.421
1.050	.267	.277	.288	.298	.308	.318	.329	.339	.349	.359	.370	.380	.390	.401	.411	.421	.431
1.075	.273	.284	.294	.305	.315	.326	.336	.347	.357	.368	.378	.389	.399	.410	.421	.431	.442
1.100	.280	.290	.301	.312	.323	.333	.344	.355	.366	.376	.387	.398	.409	.419	.430	.441	.452
1.125	.286	.297	.308	.319	.330	.341	.352	.363	.374	.385	.396	.407	.418	.429	.440	.451	.462
1.150	.292	.304	.315	.326	.337	.348	.360	.371	.382	.393	.405	.416	.427	.438	.450	.461	.472
1.175	.299	.310	.322	.333	.345	.356	.367	.379	.390	.402	.413	.425	.436	.448	.459	.471	.482
1.200	.305	.317	.328	.340	.352	.364	.375	.387	.399	.410	.422	.434	.446	.457	.469	.481	.493
1.225	.311	.323	.335	.347	.359	.371	.383	.395	.407	.419	.431	.443	.455	.467	.479	.491	.503
1.250	.318	.330	.342	.354	.366	.379	.391	.403	.415	.427	.440	.452	.464	.476	.488	.501	.513
1.275	.324	.336	.349	.361	.374	.386	.399	.411	.423	.436	.448	.461	.473	.486	.498	.511	.523
1.300	.330	.343	.356	.368	.381	.394	.406	.419	.432	.444	.457	.470	.483	.495	.508	.521	.533
1.325	.336	.349	.362	.375	.388	.401	.414	.427	.440	.453	.466	.479	.492	.505	.518	.531	.544
1.350	.343	.356	.369	.382	.395	.409	.422	.435	.448	.461	.475	.488	.501	.514	.527	.541	.554
1.375	.349	.363	.376	.389	.403	.416	.430	.443	.456	.470	.483	.497	.510	.524	.537	.550	.564
1.400	.355	.369	.383	.396	.410	.424	.437	.451	.465	.478	.492	.506	.519	.533	.547	.560	.574

HOW TO OPERATE THE EQA™ EMULSION QUALITY ANALYZER

DILUENT SOLUTION PREPARATION

Use care when preparing the diluent solution. Dilute one bottle of EQA™ Solution Concentrate. **CAUTION: The solution is caustic and appropriate care should be taken during its use (see bottle label).**

A. Remove the screw cap. Use a sharp instrument to slit the membrane three-quarters of the way around inside the neck, leaving the remainder of the membrane attached to the rim of the bottle.
B. Pour the contents through a glass funnel into a one-liter, glass-stoppered, volumetric flask. Use care to avoid loss of concentrate. If a volumetric flask is not available, 4 fillings of the 250 mL graduated cylinder can be used.
C. Rinse the residue from the empty bottle and membrane by filling with distilled water and pouring the contents into the flask. Repeat 4 times.
D. Dilute the flask contents to exactly one liter, using distilled water, and mix well.
E. The diluent solution can be stored in an airtight plastic container, so that the remainder of it can be used in future testing.

SAMPLE PREPARATION

NOTE: Extreme care should be used in all of these volume measurements to assure accurate results!

A. Accurately determine the percentage of butterfat content in a sample taken from the batch of milk to be homogenized. Ideally, the sample should be collected from the processing line just before or after the homogenizer.
B. Using the graduated cylinder supplied, place exactly 250 mL of distilled water into a clean 400 mL beaker or other suitable container.
C. Using the graduated cylinder supplied, add exactly 10 mL of the diluent solution to the 250 mL of distilled water in the beaker.
D. Warm the milk sample to 80 to 100°F and, using the micro-pipette supplied, pipette exactly 1 mL of this sample into the beaker.
E. Stir the contents vigorously.

ABSORBANCE INDEX MEASUREMENT

A. Before turning on the EQA, be sure that the instrument is located so that the air vents in the rear of the unit are not blocked.
B. Although the EQA will not be damaged should the instrument be left on over extended periods of standby, the life of the light-source lamp will be shortened, and slow heat buildup could cause instability of readings. Best practice is to turn off the EQA after an hour so of idle standby.
    1. Turn on the EQA and allow at least ten minutes for warm-up.
    2. Depress the TEST button to ensure that the display is functioning properly. A readout of -1.888 should appear in the display window. If the display does not read -1.888, the instrument should not be used and must be returned to the Factory for service.
    3. Fill one of the sample cells with distilled water. For accurate results the glass surfaces of the sample cell must be absolutely clean and dry. Using a lens tissue, Kim-wipe or equivalent, clean and dry the glass surfaces, in order to ensure that fingerprints and other foreign matter are removed (see label on sample cell case). These cells should always be stored, clean and dry, in their protective case to prevent damage to the precision surfaces.
    4. Insert cell into the SAMPLE compartment, aligning frosted glass surfaces so that one of the frosted surfaces faces the operator. (If cell is inserted improperly, a totally erroneous reading, such as 1.500 or larger, will result.) Close compartment door and "zero" the instrument, using ZERO ADJUST knob, so that the display reads .000.
    5. Fill second sample cell with diluted milk sample and insert into compartment in place of distilled water reference cell. (Be sure cell has been cleaned and aligned, as above.) If several samples are to be tested, the "zero" should be checked before each test, and sample cell should be thoroughly rinsed and dried between samples.
    6. The absorbance index reading will automatically appear in the display window, after the SAMPLE compartment door is closed. The reading should be noted immediately, because it may drift if allowed to stand for several minutes before reading.

AVERAGE FAT GLOBULE DIAMETER DETERMINATION

A. Locate the vertical column on the chart, which refers to the pre-determined percentage of butterfat in the sample. For greatest accuracy, "in-between" fat percentages can be interpolated when using the chart.
B. Read down this column to find the absorbance index number closest to that indicated by the EQA readout. Again, in-between numbers can be interpolated for better accuracy.
C. Move horizontally all the way to the left and read the average fat globule diameter in micrometers. This number is a direct indication of creaming rate and emulsion quality.

TYPICAL PRACTICE EXAMPLE

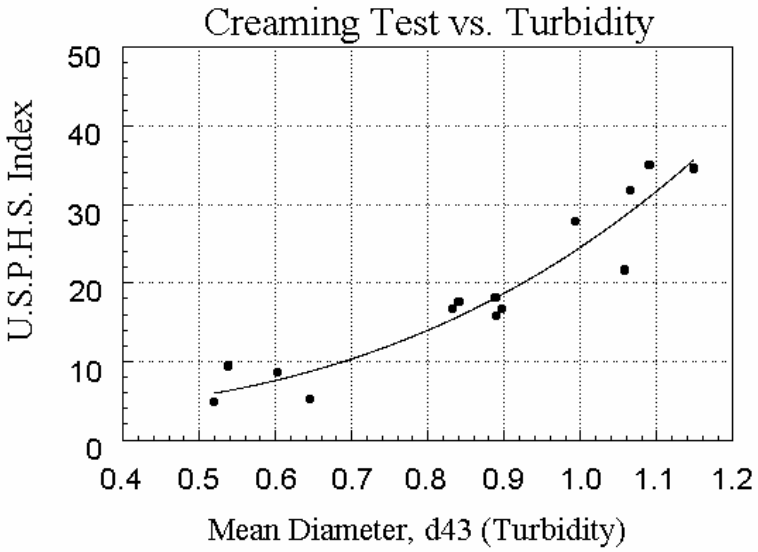
A. A milk sample having a 3.10% butterfat content was prepared and inserted into the EQA. An absorbance index reading of 0.281 was displayed.
B. Locate the 3.10% butterfat column on the Absorbance Index Chart. Read down the column to find the absorbance index 0.281.
C. Move all the way to the left to find the average fat globule diameter - 0.925 micrometers.

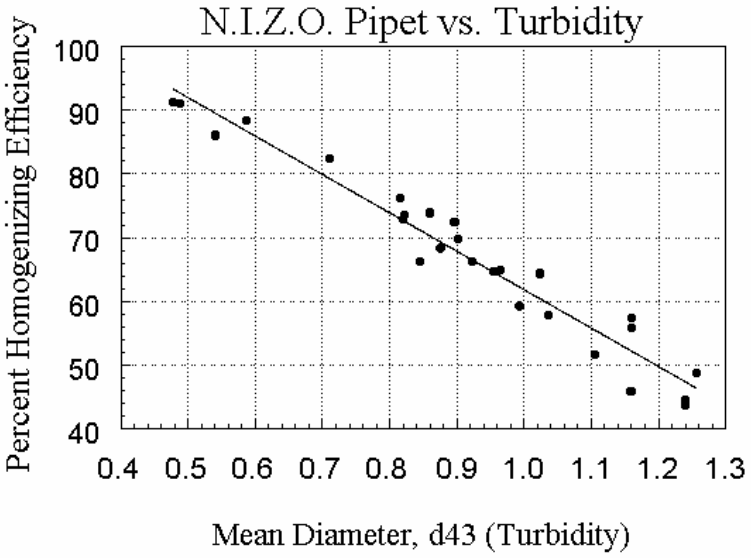
ABSORBANCE INDEX CHART II

For oils with refractive index in the range of 1.470 – .1482 (25°) - (not for use with butterfat)

Percentage of Oil in Sample

Average Oil Globule Diameter	2.50	2.60	2.70	2.80	2.90	3.00	3.10	3.20	3.30	3.40	3.50	3.60	3.70	3.80	3.90	4.00
0.300	.035	.036	.038	.039	.041	.042	.043	.045	.046	.048	.049	.050	.052	.053	.055	.056
0.325	.052	.054	.056	.058	.060	.062	.064	.066	.068	.070	.072	.074	.076	.078	.081	.083
0.350	.067	.070	.072	.075	.078	.080	.083	.086	.088	.091	.094	.096	.099	.102	.105	.107
0.375	.081	.085	.088	.091	.094	.098	.101	.104	.107	.111	.114	.117	.120	.124	.127	.130
0.400	.095	.098	.102	.106	.110	.114	.117	.121	.125	.129	.133	.136	.140	.144	.148	.151
0.425	.107	.112	.116	.120	.124	.129	.133	.137	.142	.146	.150	.154	.159	.163	.167	.172
0.450	.119	.124	.129	.133	.138	.143	.148	.152	.157	.162	.167	.171	.176	.181	.186	.191
0.475	.130	.135	.141	.146	.151	.156	.162	.167	.172	.177	.182	.188	.193	.198	.203	.208
0.500	.141	.147	.152	.158	.163	.169	.175	.180	.186	.192	.197	.203	.209	.214	.220	.225
0.525	.151	.157	.163	.169	.175	.181	.187	.193	.199	.205	.211	.217	.224	.230	.236	.242
0.550	.161	.167	.174	.180	.186	.193	.199	.206	.212	.219	.225	.231	.238	.244	.251	.257
0.575	.170	.177	.183	.190	.197	.204	.211	.217	.224	.231	.238	.245	.251	.258	.265	.272
0.600	.179	.186	.193	.200	.207	.214	.222	.229	.236	.243	.250	.257	.264	.272	.279	.286
0.625	.187	.195	.202	.210	.217	.225	.232	.240	.247	.255	.262	.270	.277	.284	.292	.299
0.650	.195	.203	.211	.219	.227	.234	.242	.250	.258	.266	.273	.281	.289	.297	.305	.312
0.675	.203	.211	.219	.227	.236	.244	.252	.260	.268	.276	.284	.292	.301	.309	.317	.325
0.700	.211	.219	.227	.236	.244	.253	.261	.270	.278	.286	.295	.303	.312	.320	.329	.337
0.725	.218	.227	.235	.244	.253	.261	.270	.279	.288	.296	.305	.314	.323	.331	.340	.349
0.750	.225	.234	.243	.252	.261	.270	.279	.288	.297	.306	.315	.324	.333	.342	.351	.360
0.775	.232	.241	.250	.260	.269	.278	.287	.297	.306	.315	.324	.334	.343	.352	.361	.371
0.800	.238	.248	.257	.267	.276	.286	.296	.305	.315	.324	.334	.343	.353	.362	.372	.381
0.825	.245	.254	.264	.274	.284	.294	.303	.313	.323	.333	.343	.352	.362	.372	.382	.391
0.850	.251	.261	.271	.281	.291	.301	.311	.321	.331	.341	.351	.361	.371	.381	.391	.401
0.875	.257	.267	.277	.288	.298	.308	.319	.329	.339	.349	.360	.370	.380	.390	.401	.411
0.900	.263	.273	.284	.294	.305	.315	.326	.336	.347	.357	.368	.378	.389	.399	.410	.420
0.925	.268	.279	.290	.301	.311	.322	.333	.344	.354	.365	.376	.386	.397	.408	.419	.429
0.950	.274	.285	.296	.307	.318	.329	.340	.351	.362	.373	.383	.394	.405	.416	.427	.438
0.975	.279	.290	.302	.313	.324	.335	.346	.358	.369	.380	.391	.402	.413	.425	.436	.447
1.000	.285	.296	.307	.319	.330	.341	.353	.364	.376	.387	.398	.410	.421	.433	.444	.455
1.025	.290	.301	.313	.324	.336	.348	.359	.371	.382	.394	.406	.417	.529	.440	.452	.463
1.050	.295	.306	.318	.330	.342	.354	.365	.377	.389	.401	.413	.424	.436	.448	.460	.471
1.075	.300	.312	.323	.335	.347	.359	.372	.383	.395	.407	.419	.431	.443	.455	.467	.479
1.100	.304	.316	.329	.341	.353	.365	.377	.390	.402	.414	.426	.438	.450	.463	.475	.487
1.125	.309	.321	.334	.346	.358	.371	.383	.395	.408	.420	.433	.445	.457	.470	.482	.494
1.150	.314	.326	.339	.351	.364	.376	.389	.401	.414	.426	.439	.451	.464	.477	.489	.502
1.175	.318	.331	.343	.356	.369	.382	.394	.407	.420	.432	.445	.458	.471	.483	.496	.509
1.200	.322	.335	.348	.361	.374	.387	.400	.413	.425	.438	.451	.464	.477	.490	.503	.516
1.225	.327	.340	.353	.366	.379	.392	.405	.418	.431	.444	.457	.470	.483	.496	.510	.523
1.250	.331	.344	.357	.370	.384	.397	.410	.423	.437	.450	.463	.476	.490	.503	.516	.529
1.275	.335	.348	.362	.375	.388	.402	.415	.429	.442	.455	.469	.482	.496	.509	.522	.536
1.300	.339	.352	.366	.380	.393	.407	.420	.434	.447	.461	.474	.488	.502	.515	.529	.542
1.325	.343	.357	.370	.384	.398	.411	.425	.439	.453	.466	.480	.494	.507	.521	.535	.549
1.350	.347	.361	.374	.388	.402	.416	.430	.444	.458	.472	.485	.499	.513	.527	.541	.555
1.375	.351	.365	.379	.393	.407	.421	.435	.449	.463	.477	.491	.505	.519	.533	.547	.561
1.400	.354	.368	.383	.397	.411	.425	.439	.453	.468	.482	.496	.510	.524	.538	.553	.567





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