Statistical Methods for Comparing GPC Curves

LÁSZLÓ FÜZES, *Research Institute for the Plastics Industry, H-1950 Budapest, Hungary*

Synopsis

The possibilities of comparing the GPC curves of two or more polymers were studied, and the method of "distinguished points" (DPs) is suggested for this purpose. The features of these DP values as random variables were investigated. According to the experimental results they meet the requirements of the statistical tests applied in this text. In order to indicate the significant deviation or the agreement of the DP values of GPC curves of two polymers, the sequential U and t tests are suggested, because with these methods the number of the necessary parallel measurements is considerably decreased, and one can also decide on the magnitude of the variation one must be able to detect, while in the case of more than two polymers the methods of the analysis of variance can be utilized. The molecular weight ranges in which significant differences occur can also be determined. The described methods were tested by materials of known molecular weight distributions.

INTRODUCTION

It is very important to know the reproducibility of data measured by gel permeation chromatography (GPC), that is, to know if the observed differences between the molecular weight distributions (MWD) of the investigated polymers are due to real deviations or only to experimental error. In certain cases the knowledge of differences might be more important than that of the "absolute" values.

The goal of this study was to develop a practical method for comparing MWD curves of polymers measured by the same GPC instrument. In order to use the methods of mathematical statistics for this purpose, it was necessary to investigate (1) which parameters are representative of molecular weight distribution, and (2) the properties of these parameters as random variables, that is, if they follow the normal (Gaussian) distribution, and the factors having an effect on their standard deviations. The symbols most frequently used in this text are given in the Appendix.

EXPERIMENTAL

Two GPC systems equipped with differential refractometer detectors were used in this study. One was a Waters GPC-200 set (GPC A) working at 130°C with 1,2,4-trichlorobenzene as solvent. Five Styragel columns (Waters Assoc., U.S.A.) of nominal porosities 6.5×10^4, 2.5×10^4, 9×10^3, 10^3, and 2.5×10^2 Å, respectively, were connected in series. The second instrument (GPC B) was assembled in our laboratory and was operated at room temperature using tetrahydrofuran as the solvent. This instrument utilized three Styragel columns (Waters Assoc.) of nominal porosities 10^5, 10^4, and 3×10^3 Å, respectively.

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The flow rate was kept 1 ml/min, and the concentration of the injected samples was 1 g/l. for the standard fractions (see Table I) and 5 g/l. for industrial samples for both instruments. One count represents 5 ml for GPC A and 1.67 ml for GPC B. The characteristics of the polystyrene samples used in this work are given in Table I. The measurements were evaluated by a Hewlett–Packard 9830 computer.

THEORETICAL

Sources of Experimental Error

Differences between the MWD curves of polymers are caused mostly by real deviations in the MWDs, sampling errors, environmental influences, and errors in data evaluation. In order to avoid systematic errors, the subsequent runs of the investigated polymers should be carried out in the shortest possible time and in random order.

The errors in data evaluation can be reduced by using the normalized chromatograms instead of the MWD curves, which are the results of sophisticated mathematical procedures (e.g., correction for spreading).

Possibilities for Comparing GPC Measurements

A comparison of GPC chromatograms is a rather difficult procedure because we know only an optional number of coordinates of the curves (their shapes), but a GPC curve cannot be considered as a histogram of random variables. Two GPC chromatograms can be regarded equivalent if they do not deviate from each other (at any point) more than can be explained by experimental errors. If we want to investigate differences between GPC curves, a certain number of variables representing the whole chromatogram should be defined. Efficient statistical methods require the variables in question to be random and independent, following the normal (Gaussian) distribution.[1]

The simplest way is to compare the number- and weight-average molecular

TABLE I
Characterization of Samples

Sample	Number of parallel measurements		$\overline{M}_n$	$\overline{M}_w$	Supplier
	GPC A	GPC B			
Styropor	17	23	96,000	220,000	BASF
I	—	—	773,000	867,000	Waters Assoc.
II	—	10	640,000	670,000	
III	—	7	193,000	200,000	
I + III[a]	7	—	250,000	485,000	
IV	7	11	20,200	20,800	
V	—	7	3,100	4,000	
VI	7	11	1,950	2,100	
Squalan	12	13	421	421	REANAL (Hungary)
Lustrex	—	3	79,000	235,000	Monsanto Chem.
706	—	3	136,000	258,000	National Bureau of Standards

[a] Mixture of samples I and III.

weights as well as the second moment of the MWDs. Unfortunately, on the basis of the investigation of polymers in Table I, it could not be proved that these quantities as random variables were normally distributed (Appendix B). This means that the comparison of these variables by the help of the generally used statistical methods is probably incorrect.

As a practical way for comparing GPC chromatograms, the method of "distinguished points" (DP) is suggested. This method involves the selection of properly definitive DPs of the curves. An optimal number of these "distinguished points" is to be compared by statistical methods.

The standard deviation of the DPs has to be measured experimentally for each GPC instrument. The points determined by the 10%, 30%, 50%, 70%, and 90% values of the integral curves of GPC chromatograms are suggested as optimal DPs for the following reasons:

a. On the one hand, if the number of DPs is too small, the fact that there is no significant difference in the DP values is not very convincing evidence of the equivalence of the MWDs. On the other hand, the probability of the error of type I for the combination of the decisions at each DP (the value of α^*) is increasing with the number of DPs (N) according to Eq. (1), if the decisions are uncorrelated:

$$\alpha^* = 1 - (1 - \alpha)^N \tag{1}$$

where α is the probability of the error of type I for a single decision (see Appendix). The probability of error of type II (β) will certainly decrease with N, but it is sufficient to know the value for which the actual β is smaller, and so we can use the original β value. It seems that if in the case of real polymers there is no significant difference in the positions of the suggested five DP values, it is convincing enough for the equivalence of the MWDs, while at the same time the value of α^* is not increasing to an intolerable degree.

b. The DP values measured at the same per cent values of different chromatograms are certainly independent random variables when compared to each other. As for the DP values measured at different per cent values of the same chromatogram, this cannot be stated, for they are, especially in the case of polymers having a narrow MWD, more or less correlated. This means only that the α^* values will be smaller than the computed ones—because eq. (1) is valid only for uncorrelated decisions—and during the investigation of the statistical properties of the DP values only one of the five points has to be taken into consideration for each polymer.

c. Having applied the test of Shapiro and Wilk[2–4] to the DPs of the chromatogram of the samples in Table I, it was found that the DPs followed the normal (Gaussian) distribution with a probability of higher than 97% (DF = 114 was obtained because for each polymer only one of the DP values was randomly selected).

d. No significant tendency in the standard deviation values was detected for the two GPC system (Bartlett test[5]). Hence, the standard deviation of the DP values can be regarded as constant, independent of molecular weight and of the shape of the chromatograms for a given GPC instrument.

Statistical Methods

In order to compare the DP values of the investigated polymers, the following methods of mathematical statistics are suggested: (a) for the investigation of two samples, the sequential U test,[1,6] if the standard deviation of DP's is known; if the standard deviation is not known, the sequential t test[1] can be used. (b) For more than two polymers, the methods of analysis of variance[1] can be applied.

Comparison of Two Polymer Samples

To perform sequential U and t tests, there is no need to decide beforehand the number of parallel measurements for a given α, β, and δ set of data (see Appendix) like in the case of the "classical" U and t tests. After each measurement we have to compute a simple decision function, and if it exceeds certain critical values, no more measurement is needed and a decision corresponding to α and β can be made. In the case of the sequential methods, the expected value of the number of parallel measurements is generally only half of that of the "classical" methods.

In order to determine the accurate value of the standard deviation of a random variable (σ), one has to perform 1 or 200 measurements at least; but considering the advantages of the knowledge of σ it is worthwhile doing so. Of course, parallel measurements performed for other purposes can also be utilized for computing σ.

The most convenient way to perform sequential tests is a graphic procedure, as follows. In the case of the sequential U test,

$$h_0 = -h_1' = -\frac{2\sigma^2}{\delta} \ln\left(\frac{1 - 0.5\alpha}{\beta}\right) \tag{2}$$

and

$$h_1 = -h_0' = \frac{2\sigma^2}{\delta} \ln\left(\frac{1 - \beta}{0.5\alpha}\right) \tag{3}$$

When selecting the value of α we have to take into consideration that α^* can be computed by eq. (1).

Then, let us calculate the expected sample number, which is

$$C = -h_1 h_0 / 2\sigma^2 \tag{4}$$

or less. If C is too large, modify the value of α or β, or of δ. Prepare the plot according to Figure 1, where $S = \delta/2$ and n is the number of parallel measurements.

Calculate the actual DP values for the investigated polymers (A and B): T_{Aij} and T_{Bij} ($i = 1, 2, \ldots, n$ and j = 10%, 30%, 50%, 70%, and 90%), i.e., the elution volumes at the distinguished per cent values of the integral curves of the chromatograms. After every runs of polymers A and B, compute for each i and j

$$\Delta T_{ij} = T_{Aij} - T_{Bij} \tag{5}$$

Then let us summarize for i the ΔT_{ij} values in the case of each j:

$$\sum_{i=1}^{n} \Delta T_{ij}$$

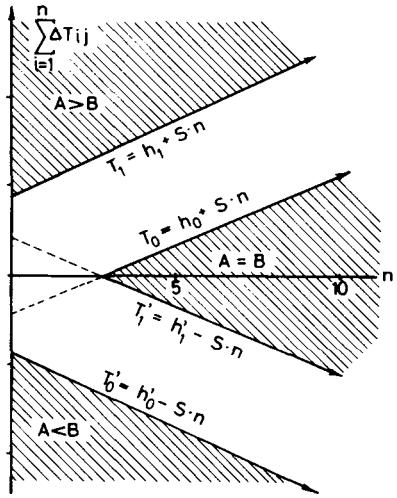

Fig. 1. Schematic decision plot for sequential U tests.

and plot the results. If after the nth measurement (1) in the case of every j the summarized values are located in the area A = B; (2) at least one of them is in the area of A > B or in the area A < B, then the test is finished and a decision is to be made as follows: (1) The MWDs of the investigated polymers are equivalent or at least the difference between them is less than δ (the risk of this decision is β), (2) there is at least δ difference between the MWDs (the risk of this decision is α^*). If none of these two cases is fulfilled, a new pair of measurements has to be performed.

If the value of σ is unknown, the sequential t test is to be used for comparing

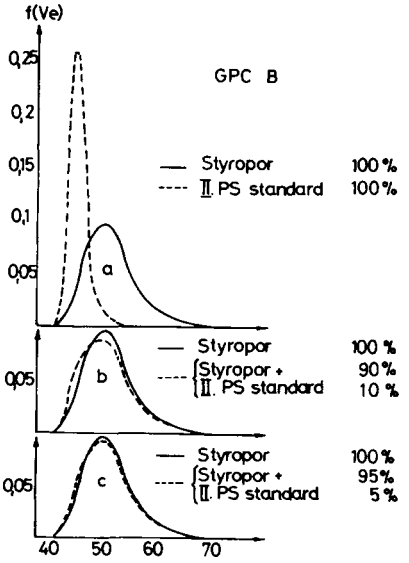

Fig. 2. Expected chromatograms of the pure polymers and mixtures: (a) pure Styropor and pure PS II standard; (b) pure Styropor and mixture of 90% Styropor and 10% PS II standard; (c) pure Styropor and mixture of 95% Styropor and 5% PS II standard.

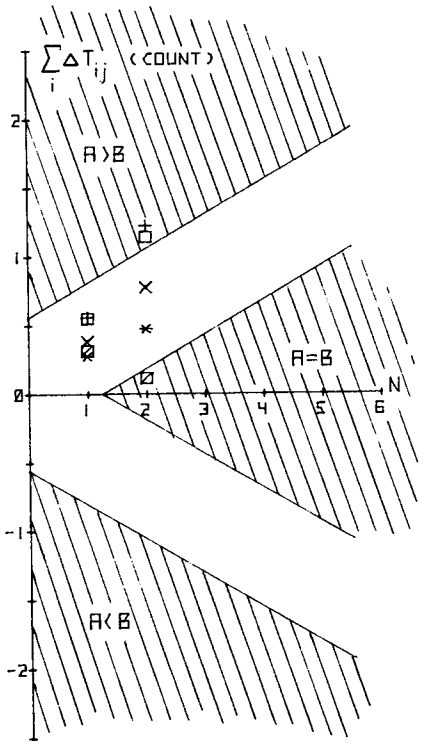

Fig. 3. Sequential U test for Example I. Cumulative differences of DP values ($\sum_i \Delta T_{ij}$) concerning 10% (+), 30% (□), 50% (x), 70% (*), and 90% (⧄) of the integral curves of the chromatograms as function of the number of consecutive pairs of measurements (N).

two polymers. The procedure is very similar to that of the sequential U test, but the boundaries of the decision areas are curves instead of straight lines here. The points of these curves are tabulated.[1] The other difference is that the

$$\sum_{i=1}^{n} \Delta T_{ij} \Big/ \left(\sum_{i=1}^{n} \Delta T_{ij}^2 \right)^{0.5}$$

values are to be plotted. Unfortunately, the sequential t test needs at least four parallel measurements to prove a significant difference, and so it can only be suggested for comparing GPC chromatograms until the determination of the accurate value of σ.

Comparison of More Than Two Polymer Samples

The DP values of more than two polymers can be compared by the methods of analysis of variance.[1] Each polymer has to be measured at least twice. The parallel runs should be carried out in random order and in the same number if possible (it gives the maximal information for a given number of runs and makes the calculation easier).

Let the number of polymers be z and the number of parallel runs for the kth polymer be n_k ($k = 1, 2, \ldots, z$). Determine the actual DPs for each chromatogram: T_{kij} ($i = 1, 2, \ldots, n_k$, j = 10%, 30%, 50%, 70%, 90%). Calculate for each j the F_j values according to equations in Appendix C.

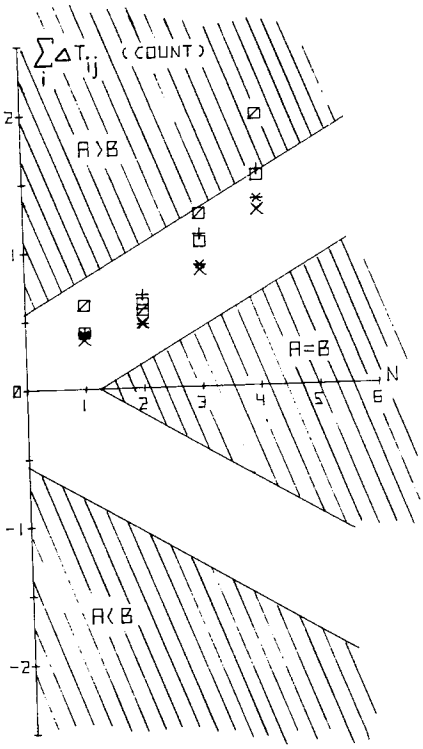

Fig. 4. Sequential U test for Example II. Cumulative differences of DP values ($\sum_i \Delta T_{ij}$) concerning 10% (+), 30% (□), 50% (x), 70% (*), and 90% (⧄) of the integral curves of the chromatograms as function of the number of consecutive pairs of measurements (N).

Having carried out the calculations for every j, compare the actual F_j values to the critical one obtained from tables according to the degrees of freedom of the S_{II}^2 and S_I^2 values.

The $\alpha = 0.01$ values are suggested when using the tables of critical F values because α^* is 0.05 from eq. (1) in this case. The magnitude of β cannot be taken into consideration because it is given only for the ratio of the S_{IIj}^2 and S_{Ij}^2 values which cannot be interpreted in terms of differences in molecular weight.

If the F_j valûes (1) are all smaller or (2) at least one of them is greater than the critical one, we can decide that (1) on the basis of the actual measurements no significant differences were found; (2) a significant difference exists between the MWDs of the investigated polymers (at least one of them deviates from the others). The risk of this decision is α^*.

This method can prove only the existence of differences; no quantitative estimation of magnitude of the deviations is given. Yet by increasing the number of parallel measurements we can disclose smaller differences. In order to estimate the actual differences, however, it is possible to refer to the average molecular weights of the investigated samples.

EXAMPLES

These methods are demonstrated by experiments where the chromatograms of polymers that have broad and only slightly different MWDs are compared. The results are more convincing if the differences in question are known *a priori.*

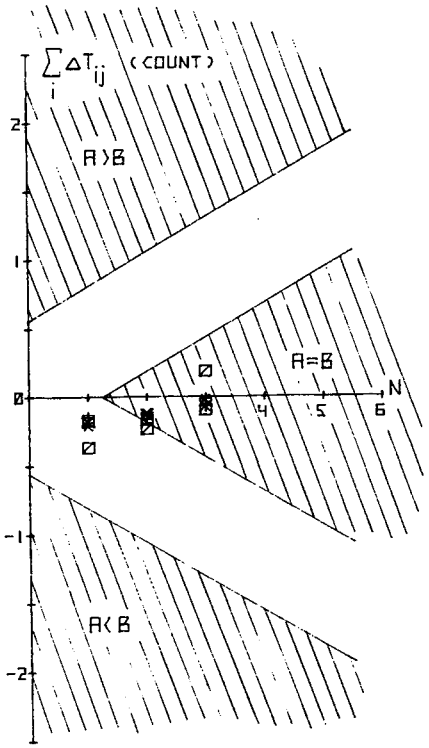

Fig. 5. Sequential U test for Example III. Cumulative differences of DP values ($\sum_i \Delta T_{ij}$) concerning 10% (+), 30% (□), 50% (x), 70% (*), and 90% (⧄) of the integral of the chromatograms as function of the number of consecutive pairs of measurements (N).

These conditions exist if we compare a mixture of two polymers of known MWDs to one of the components. The sequential U test is demonstrated by the following three examples (see Table I): (I) Styropor and 90% Styropor plus 10% polystyrene sample II; (II) Styropor and 95% Styropor plus 5% polystyrene sample II; (III) as a check of the method, the samples containing 95% Styropor plus 5% polystyrene sample II are compared to each other, that is, the chromatogram of the first run is compared to that of the second one, and so on.

The MWDs of the above-mentioned two mixtures and the pure Styropor are compared by analysis of variance as shown in Example IV.

The chromatograms in question are shown in Figure 2, where the expected curves for the pure Styropor and the II polystyrene sample were obtained as an average of 17 and 10 parallel measurements, while the expected chromatograms of the mixtures were computed by the linear combination of the curves of the pure samples.

The plots of the graphically performed tests are shown in Figures 3–5, while the computations for Example I and Example IV are tabulated in Tables II and III.

TABLE II
Calculation for Sequential U Test, Example I[a]

	$i = 1$				$i = 2$			
j, %	T_{Aji}, counts	T_{Bji}, counts	ΔT_{ji}, counts	$\sum_i \Delta T_{ji}$, counts	T_{Aji}, counts	T_{Bji}, counts	ΔT_{ji}, counts	$\sum_i \Delta T_{ji}$, counts
10	45.420	44.860	0.560	0.560	45.521	44.855	0.666	1.226
30	48.022	47.471	0.551	0.551	48.039	47.562	0.591	1.142
50	50.071	49.686	0.385	0.385	50.050	49.658	0.392	0.777
70	52.304	52.027	0.277	0.277	52.197	51.998	0.199	0.476
90	56.579	65.262	0.317	0.317	56.239	56.440	−0.201	0.116

[a] Measured DP values (T_{Aji}, T_{Bji}) and their differences (ΔT_{ji}) as used for a sequential U test in Example II. A, pure Styropor, B, mixture.

TABLE III
Results of Comparing Pure Styropor (k = 1), Mixture of 90% Styropor and 10% PS Sample II (k = 2), and Mixture of 95% Styropor and 5% PS Sample II (k = 3)[a]

	j = 10%	30%	50%	70%	90%	k	i
T_{ijk}, counts	45.420	48.022	50.071	52.301	56.579	1	1
	45.521	48.039	50.050	52.197	56.239		2
	45.395	48.030	50.119	52.455	57.052		3
	45.401	47.992	50.053	52.336	56.765		4
	45.531	48.138	50.226	52.547	57.162		5
	45.571	48.168	50.228	52.501	56.986		6
	44.860	47.471	49.686	52.027	56.262	2	1
	44.855	47.448	49.658	51.998	56.440		2
	44.982	47.618	49.758	52.056	56.440	3	1
	45.129	47.786	49.937	52.266	56.807		2
	45.100	47.696	49.848	52.123	56.407		3
	45.112	47.695	49.797	52.029	56.273		4
	45.169	47.759	49.860	52.146	56.592		5
	45.076	47.685	49.800	52.044	56.170		6
B_{jk}, counts	45.473	48.065	50.125	52.390	56.797	1	
	44.858	47.460	49.672	52.013	56.351	2	
	45.095	47.707	49.833	52.111	56.448	3	
C_j, counts	45.223	47.825	49.935	52.216	56.584		
S_{Ij}^2, count^2	0.004	0.004	0.005	0.012	0.079		
S_{IIj}^2, count^2	0.371	0.348	0.208	0.166	0.246		
F_j	82.48	89.30	41.78	14.14	3.11		

[a] By analysis of variance in Example IV. $DF_{II} = 2$; $DF_I = 11$; $F_{critical} = 7.2$.

Example I

In the first case (Fig. 3), the value of $\Sigma_i \Delta T_{ij}$ exceeded the critical values at j = 10% and at j = 30% (see Table II) after the second pair of parallel measurements. Since the points were located in the area A > B (where A means pure Styropor and B, the mixture), it could be stated with a risk of 5% (α^* = 0.05) that the MWD of the mixture was shifted to the higher molecular weights (to the lower elution volumes) at the high molecular weight part. As the first derivative of the log M–V_e (calibration) curve was 0.087 (count^{-1}) in this case and the value of δ was 0.5 (count), from eq. (13) the difference was 10% or more in terms of molecular weight.

Example II

The second case (Fig. 4) gave similar results, but four pairs of parallel measurements were necessary for the same decision since the differences were smaller here.

Example III

In the third case (Fig. 5), the four curves of the mixture obtained from Example II were not enough to make a decision, and so two more measurements were performed. After the third pair of runs, each $\Sigma_i \Delta T_{ij}$ value was located in the area A = B. On this basis it could be stated with a risk of 5% ($\beta = 0.05$) that the investigated samples had the same MWDs or at least the difference between them was less than 10% in terms of molecular weight.

Example IV

As can be seen from Table III, the calculated F_j values exceeded the critical one for $j = 10$, 30, 50, and 70. So we can say with a risk of 5% ($\alpha^* = 0.05$) that the MWDs of the three samples are different (at least one of them differs from the other two).

As was shown when applying the sequential tests, which also reduce the time of analysis, it is possible to decide in terms of molecular weight on the difference (δ) we think important to detect. Thus, for each statistical method in this text the molecular weight range in which the significant deviations occur can also be determined from the actual V_e values of the DPs by the help of the calibration ($\log M$–V_e) curve. These properties might be very useful, for example in quality control, where a polymer from a new batch could be compared to a standard sample or in experiments when polymers are treated in different ways.

APPENDIX

List of Symbols

α the error of type I, that is, the risk of asserting a difference (on the basis of the measurements) when none exists

α^* the error of type I for a combination of two or more similar tests when only one of these tests showing a significant difference is enough to accept the existence of a difference

β the error of type II, that is, the risk of asserting no difference when a difference of δ exists

δ the least difference one wants to detect; in the comparison of chromatograms, δ has "count" dimension. However, since we are interested in differences in terms of molecular weight, δ has to be expressed in terms of molecular weight. Since the GPC calibration curves ($\log M$–V_e) deviate only slightly from a straight line,

$$\frac{d \log M}{dV_e} \cong \frac{\Delta \log M}{\Delta V_e} = \frac{\Delta \log M}{\delta} \cong B \tag{6}$$

where M is the molecular weight, V_e is the elution volume, and B is the actual value of the first derivative of the calibration curve. It is also true then that

$$\frac{d \log M}{dM} = \frac{1}{M \ln (10)} \cong \frac{\Delta \log M}{\Delta M} \tag{7}$$

Equations (12) and (13) serve eq. (14)

$$\frac{\Delta M}{M} = \delta B \ln 10 \tag{8}$$

This means that δ is proportional to the relative differences in terms of molecular weight.

σ the standard deviation of the DP values

σ^* the standard deviation of the differences of the DP values

T_{Aij}, T_{Bij} the DP values of polymers A and B at the jth distinguished per cent value (j = 10, 30, 50, 70, and 90%) for the ith parallel measurement

T_{kij} the DP value of the kth polymer at the jth distinguished per cent value (j = 10, 30, 50, 70, and 90%) for the ith parallel measurement

$d.f.$ degrees of freedom

M_n number-average molecular weight

M_w weight-average molecular weight

m_2 second moment of the molecular weight distribution (MWD)

Test for Normality

Most statistical tests which could be efficiently used to compare, as random variables, measured quantities require the quantities in question to be normally distributed. If this condition, among others, is not fulfilled, serious errors may arise, especially when the standard deviations are investigated. Estimation of these errors is rather difficult, because it depends on the actual density function of the measured variables. In general, it can be stated[1] that the effect of nonnormality decreases as the number of parallel measurements per sample is increased in the case of statistical tests comparing the mean values of the measured variables (e.g., U and t tests). However, for tests on variances, e.g., analysis of variance, no such effect takes place.

On the other hand, in certain cases it is possible to apply an appropriate mathematical procedure[1] to the nonnormally distributed variables in order to transform them to new, normally distributed ones and perform the tests on these. In practice, a large number of parallel measurements is not available in GPC measurements (usually two to four), so it is particularly important to check for normality.

In the literature one finds many tests for this purpose, but the one developed by Shapiro and Wilk[2-4] has remarkable advantages. By the help of this method several small groups of measured data having even different expected values and standard deviations (e.g., the M_n, M_w, and m_2 values of parallel GPC measurements of different polymers) can be simultaneously tested, and so it is possible to obtain a sufficiently large number of DF values.

When investigating the reproducibility of the M_n, M_w, and m_2 values of polymers in Table I, it was found that even if the above-mentioned transformation, in this particular case a logarithmic one, was performed, the probability of their nonnormality were greater than 63% for M_n, 61% for M_w, and 79% for m_2, according to the test of Shapiro and Wilk, where the degrees of freedom were 94 in each case. This means that the normality of these variables is at least questionable, and being so they should not be used as representatives of the GPC curves in the case of statistical tests based on the condition of normally distributed variables.

Analysis of Variance

Calculate for each j the following quantities:

$$B_{kj} = \frac{1}{n_k} \sum_{i=1}^{n_k} T_{kij} \tag{9}$$

$$C_j = \sum_{k=1}^{z} \sum_{i=1}^{n_k} T_{kij} \Big/ \sum_{k=1}^{z} n_k \tag{10}$$

$$S_{Ij}^2 = \sum_{k=1}^{z} \sum_{i=1}^{n_k} (T_{kij} - B_{kj})^2 \Big/ \sum_{k=1}^{z} (n_k - 1) \qquad \mathrm{DF} = \sum_{k=1}^{z} (n_k - 1) \tag{11}$$

$$S_{IIj}^2 = \frac{\sum_{k=1}^{z} n_k (B_{kj} - C_j)^2}{z - 1} \qquad \mathrm{DF} = z - 1 \tag{12}$$

To perform F tests,[5] calculate the

$$F_j = S^2_{\text{II}j}/S^2_{\text{I}j} \tag{13}$$

values.

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