A GENERAL METHOD FOR MAKING QUANTITATIVE MICROANALYSES OF VEGETABLE DRUGS AND RELATED SUBSTANCES.*

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Several special microanalytical methods (qualitative as well as quantitative) have already been given. Attempts have been made from time to time to develop quantitative microanalytical methods which might be generally applicable, notably by Chamot, Meyer, Hanausek, Weinzierl, König, Vogel and others. These quantitative methods are largely based upon the fact that certain tissues and tissue elements are quantitatively fairly constant in plant organs and plant parts and in the various manufactured plant products found upon the market. For example, mature ginger contains a fairly definite percentage of starch. Green apples contain considerable starch, whereas ripe apples contain only a trace of starch. Belladonna roots gathered too early in the season are deficient in starch. Old thick cinnamon bark is deficient in bast. Cloves contain no sclerenchyma cells, whereas clove stems are rich in this tissue. The chief difficulty in the way of formulating definite working methods, is the lack of available data upon which to base such methods. For example, if we had a record of the starch content of ginger, of the apple, of belladonna root, of aconite root, of colchicum corm, etc., for each month of the growing season, then we could readily use the starch factor in determining the percentage of the articles gathered green or too early in the season. If we had a complete morphologically descriptive record of the development of the pollen grains in the insect flowers, we would then be in a position to determine quantitatively the amount of overripe flowers used in a given insect powder. In other instances it is possible to work out on the spot the necessary data for each quantitative analysis; as, for example, in the estimation of the percentage of stems in cloves, the percentage of black pepper refuse in black pepper, the percentage of stems in senna leaves, etc. Patience and a willingness to work are the essentials to success in the working out of such methods. As the details for the quantitative microanalysis of any one vegetable substance are fully and accurately worked out, the figures obtained should be carefully and permanently recorded for the benefit of those who follow after.

The chief source of error in making quantitative microanalytical determinations, of the kind here described, is the fact that while the different tissue elements and cell contents are fairly constant in tissues of the same kind (in the same relative position and of the same age and seasonal growth), we have no usable records of the quantitative tissue variations in plants, more especially in the perennials. For example, how does the bast, sclerenchyma and bark parenchyma in the bark of the white oak vary from year to year, not only in the trunk of the tree but also in the branches? Such a study would be of great value in the practical application of the method in the examination of cinnamon barks, sassafras bark, wild cherry bark, cascara bark and barks in general. What is the annual increase in fibrous tissue in belladonna roots? What is the relative amount of fibrous tissues in the wild growing and in the cultivated chicory? What is the relative amount of fibrous tissues in trimmed licorice roots and in the licorice trimmings? What

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is the exact quantitative relationship of like tissues in the inner and outer barks of the same species of tree? Since such data are not available, they must be carefully ascertained for each individual case or problem and the figures obtained carefully recorded.

At best any quantitative microanalytical methods which may be worked out, will give approximate results only; however, these approximate results are sufficiently accurate for all practical purposes. The chief factors in the variation of results are dependent upon the fact that the tissue elements and cell constituents vary (in different vegetable substances of the same kind gathered at the same time) in proportion to the size of the plant part or plant organ. This difference amounts to but very little in average commercial articles of the same kind and may in fact be wholly ignored. The chief reason why the microanalytical methods should be developed is because they are frequently the only means by which we may get the information desired, the chemical methods being wholly inapplicable.

The following general method is given with the hope that it will serve as a basis for a further development of this most important addition to the analytical methods employed in food and drug laboratories.

1. *Selecting the Sample.*—Secure an average commercial sample of the crude drug or spice, free from impurities. Great care must be observed in selecting and preparing an average sample. It should be a true average sample of the commercial article of recognized standard quality or grade as defined by the Department of Agriculture, Bureau of Chemistry, or in case the article is not defined or described by the Bureau of Chemistry, it must be of the quality and grade generally recognized in commerce, or as described or defined in recognizedly authoritative works of reference. Foreign inclusions, as vegetable tissues, dirt, clay, sand, pebbles, etc., must be removed by any suitable method, provided that none of the substance of the article itself is thereby removed or destroyed or rendered unrecognizable. Enough of the material must be taken to assure an average sample for analytical purposes.

In order to be able to select an average or representative sample of the commercial article, considerable experience is necessary. Spice dealers are, as a rule, excellent judges of the quality of the products to which they have given years of close observation. The wholesale drug dealers are thoroughly familiar with the appearance of the crude drugs as they are offered on the market. Grain merchants can, at a glance, determine the grades of the various cereals. Tea experts will recognize aromas and flavors entirely lost to the inexperienced observer. Fortunately, it is not expert ability which is required to select a representative sample, but rather a familiarity with a fair quality or grade of the articles to be examined. If, for example, it is desired to examine ground black pepper as to the approximate percentage of pepper hulls present, the comparison is to be made with a fair quality of whole black pepper, not with so-called "grinding peppers," or peppers otherwise defective, or with some special commercial variety or blend of black pepper. Before grinding, all sand, dirt, pepper stems, and markedly defective fruits must be removed. Considerable care must be observed in selecting an average sample of a root drug or of a drug composed of roots and rhizomes, or of leaves and stems. It is necessary to have on hand carefully selected average samples of drugs, spices, cereals, coffees, teas, cocoas, etc., inclusive of the various refuse tissues and the various milling by-products, as well as articles commonly employed as adulterants, such as nut shells, olive pits, tea dust, cocoanut shells, screenings and tailings, refuse and trimmings, colocynth seeds and rind, corn cobs, corn silk, bran and middlings, cereal chaff, wild mace, wild nutmeg, chicory, male fern chaff, tobacco stems, senna stems, senna siftings, roots of *Ruellia ciliosa*, false senega, pokeroot, etc., etc.

2. *Grinding or Powdering the Sample.*—After the average sample has been garbled, cleaned and thoroughly dried, it is reduced to a No. 80 powder, inclusive of all tissues which may be present.

In some instances it will be found necessary to reduce the material to be examined and compared, to a much greater fineness, in a special mill or a mortar. In order to make accurate starch counting possible the starch-bearing tissue must be made sufficiently fine to pass through a 200-mesh sieve or through bolting cloth. In other instances certain tissues may be separated and removed in comparative purity by means of very coarse mesh sieves (Nos. 8, 20, 40). In this manner such substances as bran, crude fiber, coarser meals, shells, etc., may be separated from the finer elements and their percentage values determined separately, as will be explained.

3. *Mixing the Powdered Sample.*—After the grinding, the entire amount must be thoroughly mixed by means of a spatula. Shaking in a container is not satisfactory. If the amount of the material is considerable (20 grammes and more), divide the thoroughly mixed material (spread out in a circular layer) into quadrants, remove one quadrant and again mix this thoroughly by means of a suitable spatula. A second division may be made if necessary. The final sample should be not less than five grammes.

4. *Making the Diluted Suspension.*—Dry the thoroughly mixed sample for one hour at a temperature of 80° C.[1] Weigh out one gramme of the sample and place it in a 25 Cc. graduated cylinder, add 5 to 10 Cc. of distilled water (or 5 to 10 Cc. of a mixture of equal parts of distilled water and glycerin) and mix thoroughly by means of a glass rod. Fill up to the 25 Cc. mark with a five percent gum acacia solution and again thoroughly mix. The gum solution will hold the tissues in suspension until the microscopic examination is made. The above makes a dilution of 1–25, which will be found satisfactory in most cases. Higher dilutions, as 1–50, 1–100, 1–250, may be used if desired, or if necessary for accurate results, as in starch counting.

Other suspending media may be used, as glycerin, oils, a thin syrup, solution of cherry gum, solution of gum mastic, gelatin solution, India gum solution, plain vaseline, etc. It is advised to stir the material in a little water first, as stated above, for if the gum solution should be added at once, considerable difficulty may be encountered in getting minute particles, starch granules in particular, evenly distributed. It is of course necessary to stir and mix the suspension thoroughly each time a new mount is to be made.

5. *Making the Counts.*—The mounts must be made without a time lapse in the entire procedure. Immediately after the thorough mixing, take up from 0.5 Cc. to 1.00 Cc. of the material by means of a graduated straight cylinder pipette, having a free outflow (1 Cc. or 2 Cc. pipettes graduated into tenths), reject 0.1 to 0.2 Cc. and then deliver just 0.2 Cc.[2] upon the counting chamber or counting slide and at once mix and spread this out between the two slips on the slide (use a platinum loop or needle, or any blunt needle, or a very thin glass rod), and cover with the special rectangular cover glass, by placing the proximal end of the cover at the distal ends of the two slips, pushing it forward upon the two slips, lying flat upon them, or lay it in place sidewise. If the exact amount indicated (namely, 0.2 Cc.) has been properly spread and the cover glass carefully placed, the entire space between the two slips and the cover glass will be occupied by the material without any excess or overflow. The mount should be set aside on a leveling table for a few minutes, otherwise starch granules may become unevenly deposited by the force of gravity.

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[1] This is not essential for practical purposes.

[2] Since the counting chambers have a uniform capacity, namely, 0.20 Cc., it is not necessary to use a pipette, simply use enough material to fill the cell.

If the powders to be examined are very fine, as starches and some meals, face powders, dusting powders, etc., the counts may be made by means of the hemacytometer, instead of the above special counting chamber. A number 80 powder does not permit the use of the hemacytometer for counting. It is perhaps self-evident that actual numerical determinations per Cc. or per gramme may be made by means of the method just outlined, since definite quantities and definite counting areas are used.

If it is desired to make careful starch counts of drugs and spices, it is necessary to use a higher degree of fineness. The particles must be small enough to pass through a 200-mesh sieve or through bolting cloth. In the case of a vegetable substance having starch aggregates, it is advised to count the aggregates as one, rather than to attempt to give the number of individual granules in each aggregate, as for example in making starch counts of oat, rice, aconite and buckwheat, but care must be observed not to omit to state this in the recorded results.

In the above method it is not necessary to give any attention to differences in specific gravity of the different tissue elements and cell contents. Should it be desired to give the percentage of sand present (by weight) in a given vegetable powder, it would be necessary to ascertain by the trial method that mixture of the substance and sand which would give the same counts as the article under examination. Let us assume that by the trial method we found that the addition of 5 grammes of sand to 5 grammes of the powder we obtained the same sand count as in the substance under examination, then we would know that the sand adulteration amounted to 50 percent. However, in the case of sand adulteration, the simpler and almost equally accurate beaker or test-tube sand test will be employed, rather than the microscope.

The depth of the mount, namely 0.2 mm., requires good working distance of the compound microscope in order that the entire depth may be brought into view. The cover glass used must be thin, not to exceed the thickness of the ordinary No. 2 covers. The usual lense combination (ocular and objective) giving a magnification of about 450 to 500 diameters may be used. A good observer who is entirely familiar with tissues and tissue elements could use a lower magnification with better effect, such as a well-corrected combination giving a magnification of about 180 diameters.

The procedure is as follows: Carefully examine each and every separate and distinct field, counting all of the characteristic tissue elements and record the findings numerically. The mechanical stage will be of advantage in shifting the counting chamber. It will be found that from thirty to fifty distinct fields can be counted in one mount. The averages of not less than fifty counts should be taken upon which to base the percentage estimates of quality or adulteration.

Before beginning the counts, it is advisable to look the mount over under the low power (about 90 diameters) for the purpose of ascertaining whether or not the material is uniformly distributed. If it is unevenly spread, a new mount should be made. In fact, the low power alone is used in examining some substances, as, for example, Cinchona bark, the identity and purity determinations of which are based upon the counting of the characteristic large bast elements. Difficulty may be encountered in getting the total counts of aggregates of cells, as bast and sclerenchyma. As a rule, however, in a number 80 powder, aggregates which are so large as to make counting difficult, are rare. Some investigators have suggested that the quantitative estimates should be based upon the actual measurements of cells and cell contents. This is not necessary, as the average of the numerical count is fully as accurate as the average based upon measurement. However, as already indicated, where identity depends upon differences in size, then measurements (linear) must be made.

The individual field counts are to be made in the following manner: In the case of rather coarse particles, as the larger bast cells, groups of sclerenchymatous tissue cells, fragments of fibrous tissue, fragments of vascular tissue, etc.,

for which the low power is usually employed, count all of the recognizable structures which lie within the circular area of the field of view, including all of the counting groups lying across the margin of the field, provided the groups or the cells extend far enough into the field of view to be distinctly recognizable. Next, shift the mount so that the new field does not reveal any of the elements of the preceding field. Unless this rule is closely observed there is likelihood of counting one cell group twice. This must be avoided. On the other hand, it is advised not to shift the mount more than is necessary to obtain an entirely new field, as above explained. The counts should be made in groups of ten, selected from five nearly equidistant areas of the mount, avoiding the extreme margins of the area of the mount, and the average of the fifty counts is taken. If the different group counts (of ten each) show wide numerical variation, it indicates that the material was not uniformly distributed on the counting chamber. The dilutions from which the counts are to be made should be such that not more than five to ten counting elements will appear in one field of view.

In making numerical counts of minute particles, such as starch granules, trichomes, epidermal cells, sparingly present and comparatively small bast and sclerenchyma cells, for which purpose the high power is usually employed, it is advisable to make the dilutions such that not more than from ten to twenty-five of the counting particles will appear in one field of view. In such cases it is the rule to count one-half of the particles which lie across the margin of the field. If the tissue elements upon which the percentage counts are to be based, are very sparingly present, from one to two or even less than one per field of view, it is necessary to take the average of many counts, 100 fields and more, in order that the average obtained may give fairly accurate percentages.

In some instances coloring agents such as phloroglucin and hydrochloric acid (lignin test), iodine test solution (for starch), and chlor-iodide of zinc, etc., will prove useful.

It is necessary that the same microscope and the same objectives and ocular be used at all times, in order that the results may be relatively uniform. For instance, should a partial count be made with one instrument, and completed with another instrument, even if approximately of the magnifying power, the results would be inaccurate. In order that the work of different analysts may be unified, it is necessary to adopt standard methods. The surface area of the field of view of each microscope used must be accurately determined by means of the stage micrometer. The Bureau of Chemistry method for the examination of tomato products directs that the field of view be unified by means of the draw tube, so that each field shall equal 1.50 sq. mm. (diameter of field of view equals 1.382 mm., therefore, area of field equals 1.50 sq. mm.). This method is faulty because no allowance is made for the differences in magnification of the microscopes used. Proper adjustment of this kind should be made by means of the ocular diaphragm, after having reduced the microscopes to the same magnification by means of the draw tube. Accurate comparative results may be obtained by any number of analysts provided a counting chamber of definite area be used. The special counting chamber recommended has a depth of 0.2 mm., the total contents of the entire area being 0.2 Cc.

6. *Making the Comparisons.*—In the identical manner as above set forth (1 to 5, inclusive), prepare and examine the article to be compared and of which the quality or purity is to be determined. From a comparison of the two averages of counts thus obtained, it is possible to determine the approximate percentage of admixture and adulteration. Let us suppose that the article in question was Cassia cinnamon and the average counts were as follows:

	The standard cinnamon.	The compared cinnamon.
Bast cells	2.5	0.8
Sclerenchyma cells	12.1	14.0
Starch granules	50.0	13.0

The most diagnostic tissue of cinnamon is the bast and if we use this count alone (2.5 : 0.8 : : 100 percent : x percent) the conclusion would be drawn that the article in question was 32 percent adulterated or 32 percent below a good or standard quality of cassia cinnamon. The other counts (sclerenchyma and starch) are corroborative. The bark parenchyma count is not given because the microscopic appearance of this tissue is non-characteristic, the cells being much broken up making counting difficult, if not impossible. The odor of the compared article was musty and the taste quite feeble. The conclusion based upon the organoleptic tests alone is that the article in question is of very inferior quality, made from old bark, and that adulteration amounts to over 30 percent.

Standard insect powder.		Compared insect powder.
Pollen grains	5.4	0.2
T-shaped or spindling hairs	2.0	2.1
Sclerenchyma cells	10.0	0.6
Fibrous tissue	8.0	60.0

The findings would indicate that the article in question was 100 percent adulterated and that the adulterant was Pyrethrum stems. The small amount of pollen found in the compared article may be wholly ignored, as that amount of pollen is normally present in and upon leaf and stem tissue, even though no flowers are present. A so-called insect powder may show abundant pollen grains and yet be 100 percent adulterated, as when foreign flowers are used, hence the analyst must be able to recognize different pollens.

Sample powdered cloves.		Clove stems.
Bast cells of clove stems	6.0	10.0
Sclerenchyma cells	12.0	21.0

In the above example the comparison is made with pure clove stems, which article is the most common adulterant of powdered cloves and in this case the two tissues, namely, bast and sclerenchyma, are present in fairly constant ratio, hence both counts may be used and should check each other, if carefully made. The double proportion would be 10 : 6 : : 21 : 12 : : 100 percent : x percent. $10x = 600 = 60$ percent; $21x = 1200 = 58$ percent. Under the law (federal pure food and drugs act) 5 percent of stems are allowed. 60 percent less 5 percent leaves 55 percent unlawful adulteration in this case. In this case the average of 60 per cent and of 58 percent, namely, 59 percent, should be taken.

Sample cocoa.		Cocoa stems.
Fragments of spiral ducts	3.8	13.9
Sclerenchyma cells	2.6	10.0

The spiral ducts are most readily recognized and are fully as diagnostic as are the sclerenchyma cells. Both tissue elements should be included in the count and the average of the two percentages obtained should be used. Thus, 13.9 : 3.8 : : 10.0 : 2.6 : : 100 percent : x percent. $13.9x = 380 = 20.1$ percent and $10x = 260 = 26$ percent. $20.1 + 26 \div 2 = 23.05$ percent of shells in the cocoa under examination. Under the law 3.50 percent of shells (called "crude fiber") is allowed, hence in the above case we have $23.05 - 3.50 = 19.55$ percent of excess of shells or crude fiber. The starch count is of little value because this substance is present in small amounts and is furthermore quite variable in amount in the different varieties of cocoa. The endosperm cell count is impracticable because the cells are very much broken up. The amount of vascular elements (represented by spiral ducts) and of sclerenchyma cells is negligible in absolutely pure (wholly freed from shells by hand) cocoa and chocolate.

In the case of aconite root, belladonna root, colchicum corm, ginger, and other plant structures rich in starch, a careful starch count would indicate whether or not the article was gathered out of season or before maturity. In the case of leaves and herbs which contain distinctive trichomes, as senna, digitalis, artemisia,

etc., these structures would serve as the basis for percentage determinations. In other cases measurements (relative size of starches in Rio and Carthagena ipecacs, relative lenghts of trichomes in India and African senna) must be resorted to in order to determine percentage admixtures.

In many instances it may not be necessary or even desirable to reduce the substance to uniform fineness. In a compound, as cattle, chicken or condition powder, one or more of the components (as bran, crude fiber, sulphur, charcoal, meals) may be separated in comparative purity by means of the nest of sieves; the several coarse and comparatively pure components are weighed and their percentage values finally computed. The finer components are mixed and reduced to the desired fineness for making the microscopic counts as already explained, and the results added to those obtained by the sifting process. The following will serve as an illustration of the method of procedure:

A condition powder, using ten grammes of a well mixed sample:

Total weight of sample	10 grammes
Bran, on coarse mesh	2.3 grammes
Corn meal, on next finer mesh	3.6 grammes
Finer remainder, on No. 60 mesh	4.1 grammes

The finer remainder reduced to a No. 200 mesh powder and examined microscopically gave the following counts (one gramme of the powder suspended in 99 Cc. of the gum solution):

Corn starch	51,000,000 per gramme
Wheat starch	8,000,000 per gramme
Sulphur particles	13,000,000 per gramme
Charcoal particles	32,000,000 per gramme
Wheat tissue elements	800,000 per gramme

In the above counts the corn starch count is to be interpreted in terms of corn meal, as no corn starch was added as such. The wheat starch count and crude fiber of wheat is to be interpreted as wheat middlings. The percentages of sulphur and of charcoal are determined by the actual trial method. The following is the reconstructed formula of the condition powder, based upon the above data.

Condition powder.

Wheat bran	25 percent
Wheat middlings	15 percent
Corn meal	40 percent
Sulphur	10 percent
Charcoal	10 percent
Total	100 percent

Corn meal reduced to a fineness so as to make a count of the total starch granules possible, gives 1,305,000,000 granules per gramme. The starch content of wheat middlings varies considerably, but an average article will give a starch count of 200,000,000 granules per gramme. One gramme of sulphur will show about 140,000,000 particles per gramme. The number of charcoal particles per gramme will depend upon the degree of fineness, in the above about 350,000,000 per gramme. From these figures it is simple to deduce the formula given.

The experienced microanalyst can estimate percentages with some degree of accuracy by optical judgment, but such estimates will not be acceptable in court proceedings. Thus, based upon the inspection of an ordinary slide mount, certain face powders are declared to contain about three percent of rice starch or corn starch, so-called arrowroot biscuits show about 2 to $2^1/_2$ percent of arrowroot starch, a pancake flour is estimated to contain 25 percent buckwheat and 75 percent wheat flour; a baking powder is estimated to contain 25 percent corn starch; etc. This is the only method possible in cases where the amount of the material is too small to permit the use of definitely weighed amounts or definite volumes.

This is clearly illustrated in the following rather extreme and highly complex example. A bit of vomit smear on a small piece of newspaper was submitted by a physician with the explanation that it represented a medicinal substance of which he desired to know the composition. A careful microscopical examination of the smear revealed the following ingredients:

1. Starch granules and the bast cells derived from the peeled roots of *Althaea officinalis.*
2. Papillose epidermal cells with red coloring matter of the petals of the red rose.
3. Abundant globules of metallic mercury.
4. Numerous chalk particles.
5. A few pollen grains.
6. Crystals of calomel.
7. Abundant crystals of basic mercuric sulphate (turpeth mineral).
8. The starch granules, yellow lignified fibers and the crystal bearing fibers of *Glycyrrhiza glabra.*

As stated in the introduction, one of the most important requirements or qualifications on the part of the microanalyst, is the ability to interpret the finding intelligently and correctly. The above findings are not numerous nor were they difficult to recognize. The interpretation proved very interesting, as follows:

(*a*) Confection of Rose, U. S. P. indicated by (2) and (5). Turning to the Pharmacopoeia, the formula for confection of rose was copied.

Red rose petals (No. 60 powder)	80 grammes
Sugar	640 grammes
Clarified honey	120 grammes
Stronger rose water	160 Cc.

(*b*) Blue Mass, U. S. P., indicated by (1), (3) and (8).

Mercury	35 grammes
Licorice powder	5 grammes
Althaea powder	25 grammes
Glycerin	33 Cc.
Honey of rose	34 Cc.

(*c*) Gray Powder, U. S. P., indicated by (3), (4) and (5).

Mercury	38 grammes
Clarified honey	10 grammes
Prepared chalk	57 grammes
Water, ad	100 grammes

(*d*) Calomel.

(*e*) Turpeth mineral.

Based upon the optical appearance of the several ingredients of the smear as seen under the microscope, assisted by a number of trial combinations which were compared with the smear (microscopically), the following was given as the formula of the medicinal substance represented by the material upon the bit of newspaper:

Reconstructed formula based upon the above.

Confection of rose, U. S. P.	8 parts
Blue mass, U. S. P.	12 parts
Gray powder, U. S. P.	35 parts
Calomel	15 parts
Turpeth mineral	30 parts

Naturally, problems of the kind just illustrated require much time and careful study, and as already indicated, much experience in the practical use of the compound microscope.

The following quantitative microanalytical determinations should be made by the student under the direction and guidance of the instructor. Each problem

will require at least one full laboratory period. The exercises are graded, beginning with a few simple percentage determinations of simple powders, the microscopical characteristics of which are easily recognized, and ending with the percentage determination of a compound powder. By the time the student has worked out the ten problems very carefully, he will be in a position to solve any ordinary problem in vegetable drug adulteration.

The work done by the members of the class should be compared and discussed and the differences in results should be accounted for by the instructor in charge. If the work of the students has been carefully done the percentage differences based upon the counting methods should not exceed 5. If the percentage differences exceed 5 the exercise should be repeated. The personal factor must be carefully considered. The instructor in charge must explain each problem very carefully and in great detail and no student should be allowed to begin upon the counts until it is ascertained for a certainty that he understands fully what the problem is and what the count, which he is about to make, represents. He must know why it is that only one or two tissues of each drug are selected for the purpose of making the percentage counts. The various quantitative microanalytical methods which have been proposed should be explained and discussed and the advantages and disadvantages of the several methods explained. The results of the microanalytical method herein recommended should be compared with the results of the more commonly employed chemical methods. Such a comparison will demonstrate that the percentage results by the microanalytical methods are fully as accurate as the percentage results by the usual chemical test and analyses, as carried out by analysts of about equal qualification and experience.

1. *Cinchona Bark.*—Reduce an absolutely pure average sample of *Cinchona Ledgeriana* and of *C. succirubra* to a No. 80 powder. Make suspensions in the manner already explained and by means of the special counting chamber determine the average number of bast cells per field. Take the average of not less than fifty counts of not less than two mounts. The average of the counts thus obtained represents the number of bast cells in a pure 100 percent cinchona bark. The individual bast cells should be counted. Thus a group of three bast cells should be counted as three. No attention is given to the bark parenchyma cells nor to the sparingly present sclerenchyma cells. In this particular exercise the percentage difference of the counts by the students should not exceed 1 percent.

Make percentage mixtures of cinchona bark, using absolutely pure *Rhamnus purshiana* bark as the admixture. The rhamnus bark must be reduced to the same fineness as the cinchona bark and the mixing must be thorough. Make three admixtures. One of fifty parts of cinchona bark and fifty parts of rhamnus bark; the second mixture of seventy-five parts cinchona and twenty-five parts rhamnus; the third mixture of ninety parts cinchona and ten parts rhamnus. The usual suspensions of these several mixtures are made and the counts made as for part one of this exercise. The results should be compared and discussed. Keep the admixtures for problem two.

2. *Rhamnus Purshiana Bark.*—Prepare an absolutely pure average sample of *Rhamnus purshiana* bark as for cinchona and make a count of the bast tissue, counting each group of bast cells as one. The sclerenchyma cells (individuals and groups) are not counted as they are rather difficult to recognize and the counts based thereon would only cause confusion and would give widely varying results.

Use the mixtures prepared for the cinchona counts and from the counts obtained determine the percentages of rhamnus bark present. Do not ascertain

the rhamnus percentages by merely utilizing the counts of the cinchona bast cells as obtained from problem one. Compare the errors in the two problems from the same admixtures and it will be found that the variation in results will be somewhat greater for problem two, although the error should not exceed 3 percent, even for beginners in this kind of work.

3. *Senna Leaflets.*—Prepare two samples. One of pure whole or broken leaflets of *Cassia angustifolia* and one of pure whole or broken leaflets of *Cassia acutifolia*. Make the 1–5 suspensions and determine the number and the length of the simple single-celled trichomes present. In the *C. angustifolia* the trichomes are fewer and longer as compared with those of *C. acutifolia*. Also search for epidermal tissue fragments and note the number and size of the neighboring cells of the stomata in the two varieties of sennas.

Make a fifty to fifty admixture of the two sennas and determine the amount of each kind of senna, based upon the count of the trichomes, utilizing the following suggestion. The count of those trichomes which exceed in length the longest cells of the *C. acutifolia* represent *C. angustifolia* (100 percent). In this exercise the differences in result will be found to be considerable.

Make admixtures of each of the two samples with wheat flour and determine the amount of such admixture from the trichomatic counts. In this case the difference in the results should be slight. This particular problem will serve as a preparation for problem ten.

Make admixtures of pure senna leaflets and of senna stems and make percentage determinations; likewise of senna pods and of senna leaflets.

4. *Insect Powder.*—Secure samples of pure flower heads of the three species of pyrethrum (*Chrysanthemum roseum, C. Marshalli* and *C. androseamifolium*), both the fully expanded flower heads and the partially expanded heads, making six samples. The thoroughly dried flower heads are reduced to a No. 80 powder and the counts are made of the pollen grains (mature and immature) and of the groups of the sclerenchyma cells and also of the groups of fibrous tissue. The trichomes and the somewhat papillose epidermal cells have a diagnostic significance only. The counts thus obtained should be carefully made and the results recorded for future reference and use.

Prepare a powder of the stems of one of the species of insect flower and make a careful count of the groups of the fibrous tissue present. A few pollen grains will also be noted. The count of the groups of fibrous tissue represents a 100 percent stem tissue. Now make admixtures of pure insect powder and pure stem tissue, and from a count of pollen as well as of the stem tissue, ascertain the amount of the several admixtures. A whole series of problems will suggest itself and should be tried out as time and opportunity will permit. The sclerenchyma group count and mature pollen grain count will indicate the percentage amount of mature flower heads used. What is the number of pollen grains per gramme in a 100 percent insect powder?

5. *Digitalis Leaves.*—Secure samples of the leaves from the first year plants, and from the second year plants at the time of flowering. Dry, powder and prepare for the count as the above samples. Make counts of the simple trichomes. In the first year plants the leaves will show fewer trichomes. What are the total trichomatic counts for the first year leaf and for the second year leaf? Also make a count of the groups of fibrous tissue in the two samples of leaf.

Prepare samples of digitalis stems and make counts of the trichomes and of the groups of fibrous tissue. Make fifty to fifty admixtures of digitalis leaf and of digitalis stem and redetermine the amount of leaf and of stem, respectively, based upon the trichome count and also upon the count of the groups of fibrous tissue.

Digitalis leaf (in the powdered form) is identified by the simple and the glandular trichomes. The cells of the simple trichomes are more or less collapsed, usually one cell is flattened in one plane, the cell following being collapsed in a

plane at right angles to that of the other. The glandular trichomes usually bear two terminal secreting cells. In making the counts of the trichomes, all trichomatic fragments, distinctly recognizable as such, are to be counted as one, and it is important that all of the comparative counts be made of powders of the same degree of fineness. The percentage estimates based upon the counts of the first year and the second year leaves will be quite variable and will show wide ranges in results.

6. *Glycyrrhiza.*—Prepare samples of pure average samples of the peeled and the unpeeled licorice, and of licorice trimmings. Counts are made of the yellowish groups of fibrous tissue, especially of the groups of crystal-bearing fibers. The starch count may be ignored. In the case of the unpeeled licorice a count is to be made of the dark brown cork tissue elements. The following counts are to be made of the three samples.

a. The number of cork tissue elements and groups of such elements in the unpeeled licorice; also a count of the yellow fibrous tissue and crystal-bearing tissue groups.

b. The fibrous and crystal-bearing groups in the peeled licorice.

c. The number of fibrous tissue groups in the licorice trimmings.

Make admixtures of the pure licorice and of the licorice trimmings and determine the percentages of the admixtures from the results of the counts. The results of the counts will again be utilized in exercise ten.

7. *Sublimed Sulphur.*—Examine carefully samples of pure sublimed and of precipitated sulphur and note the microscopic differences. Make careful percentage admixtures of sublimed sulphur in plain vaseline and from such admixtures make the counts of the sulphur particles. A 1, 5 and a 10 percent mixture should be counted. If it is found that the sulphur particles are too numerous to permit counting then dilutions must be made and the percentages redetermined from a carefully prepared vaseline suspension. How many particles of sublimed sulphur in one gramme? The results of the counts are to be used in exercise ten.

8. *Black Pepper.*—Four substances are required; an average commercial sample of pure whole black pepper; an average commercial sample of whole white pepper; a sample of whole pepper known in the trade as "grinding peppers;" and a sample of "black pepper refuse" (consisting of pepper stems, tailings and screenings). These several samples are to be reduced to the same degree of fineness and the following counts made:

a. Of the black pepper, the dark to nearly black fragments and groups, consisting entirely of the pericarp tissue; and the colorless tissue fragments (starch-bearing) consisting wholly of the endosperm tissue elements. The counts represent 100 percent black pepper.

b. Of the white pepper. The dark fragments derived from the pericarp tissue are identical with those of the black pepper. They are sparingly present; and the colorless tissue fragments (starch-bearing) consisting wholly of the endosperm tissue. The two counts together represent 100 percent white pepper.

c. Of the "grinding peppers," make the counts as for black pepper, and compare with the counts for black pepper.

d. Of the black pepper refuse, make the count of the black tissue groups and the groups of fibrous tissue.

Admixture of black pepper and of black pepper refuse are to be made and the percentages of the admixtures redetermined from the counts. "Grinding peppers" will show an excess of pericarp tissue. Pepper adulterated with refuse will also show an excess of pericarp tissue, but in addition will show groups of fibrous tissue derived from pepper stems.

Admixtures of pepper and other substances which are frequently employed as adulterants, such as cereal, cornmeal, ground olive pits, etc., may be made and the percentage adulteration redetermined from the counts.

9. *Mustard.*—Three substances are required. Ground mustard (either white or black) of known purity; ground turmeric of known purity; and wheat flour. No count is to be made of the mustard, as it reveals no structure upon which a percentage count could be based. Make the following counts. A count of the yellow starch clusters of turmeric; and a count of the starch granules of the starch-bearing tissue groups of the wheat flour.

Make admixtures of mustard and of wheat flour, adding from one to five percent of turmeric, and from the counts redetermine the percentages of the admixtures.

The following addition should be made to this problem: Make counts of 100 percent ground mustard hulls, and from admixtures of mustard and of mustard hulls, redetermine the percentages of the admixtures.

10. *Compound Licorice Powder.*—This substance contains powdered sugar, the count of which can be made from an alcohol or oil mount; or, the counts of all of the ingredients may be made from a vaseline suspension. Of course it will be necessary to make a separate count of a pure sample of powdered sugar. In so doing it should be kept in mind that most of the powdered sugar of the market contains a small amount of corn starch. The amount of corn starch present should be determined according to the method given elsewhere in this paper. The following counts are to be made:

a. A count of the licorice tissue upon which the percent of licorice is based. Is the licorice of the peeled or of the unpeeled variety?

b. A count of the sulphur particles present.

c. In case anise is used as the flavoring agent, instead of the oil of anise, then a separate count must be made of a pure sample of anise in order that the percentage amount in the compound powder may be ascertained.

d. A count of the sugar particles present.

The reconstructed formula based upon the results of the several counts should be closely similar to that of the actual pharmacopoeial percentages composition.

The microscopes used by the students must be standardized to the same magnification and the same area of the field of view. In each instance it must be stated as to whether the high or the low power was used in making any given count or sets of counts. In most instances the low power will suffice. For making starch counts and counts of similar minute particles the high power must be used.

The following is a list of vegetable drugs and spices giving the microscopical characteristics of each substance named upon which the percentage counts are to be based. When more than one histological element is named, the first is the one considered the most important for the purpose of making the counts. The other elements may be used for check purposes. The structural elements proposed for purposes of making the percentage counts, are not necessarily also the more important for purposes of identification, and in many instances that combination of tissues and of tissue elements which will unmistakably identify the drug or spice, is of little value for the purpose of the percentage determination. It will be found that some vegetable drugs reveal no special identifying microscopic structures, as belladonna leaves, stramonium leaves, spigella roots and rhizomes, cypripedium roots and rhizomes, lappa roots, fennel, caraway, and others. For this very reason added adulterants are all the more readily recognized as these usually reveal some distinctive microscopical characteristic. In such cases the percentage counts are made of the adulterants and the result subtracted from the total amount, leaving the percentage of the drug or spice itself. Belladonna, for example, is

frequently adulterated with phytolacca, and the adulteration is at once recognized by the very characteristic scattering acicular crystals of phytolacca, but these diagnostic structures are of no value for making percentage determinations. On the other hand, ground olive pits, which are so frequently used for the purpose of adulterating spices, nutgall and other drugs, are not only readily recognized but are ideal for the purpose of making exact percentage counts.

LIST OF SUBSTANCES WITH MICROSCOPICAL STRUCTURES UPON WHICH THE PERCENTAGE COUNTS ARE TO BE BASED

1. Absinthium.—T-shaped trichomes.
2. Aconite leaf.—Trichomes. Sclerenchyma cells.
3. Aconite root.—Sclerenchyma cells. Starch aggregates.
4. Amygdala.—Sclerenchyma cells (for unblanched almonds only).
5. Amylum.—Size, form, markings, position of hilum, etc.
6. Anisum.—Trichomes.
7. Asclepias.—Groups of sclerenchyma cells.
8. Aspidium.—Resin particles. Starch. Scalariform ducts.
9. Aspidosperma bark.—Large sclerenchymatous bast cells.
10. Belladonna leaf.—Trichomes? Cells with microcrystalline calcium oxalate.
11. Belladonna root.—Starch? Cells bearing microcrystalline calcium oxalate? Ducts?
12. Berberis.—Sclerenchymatous bast cells.
13. Caffea.—Sclerenchymatous bast cells. Endosperm cells.
14. Calendula.—Trichomes. Pollen.
15. Calumba.—Sclerenchyma cells. Starch. Ducts.
16. Canella.—Groups of sclerenchyma cells.
17. Cannabis.—Simple trichomes.
18. Capsicum.—Color and characteristic epidermal elements.
19. Carbo.—Size and number of black particles.
20. Caryophyllus.—Bast cells. Color of tissue fragments.
21. Cascarilla.—Bast cells.
22. Chamaelirium.—Bast cells.
23. Cinchona.—Large bast cells.
24. Cinnamon.—Bast cells. Sclerenchyma cells.
25. Coca.—Fragments of the lower epidermis.
26. Colchicum corm.—Starch granules. Spiral ducts.
27. Convallaria.—Number of raphides.
28. Cornus.—Groups of sclerenchyma cells.
29. Coto bark.—Bast and sclerenchymatous groups.
30. Crocus.—Pollen grains and colored tissue.
31. Cubeb.—Groups of sclerenchyma cells and of endosperm cells.
32. Curcuma.—Number of yellow particles (agglutinated starch).
33. Cusso.—Pollen. Simple and glandular trichomes.
34. Cypripedium.—Number of raphides.
35. Dextrine.—As for starch. Recognizable starch granules.
36. Digitalis.—Non-glandular trichomes.
37. Eriodictyon.—Non-glandular trichomes.
38. Euonymus stem bark.—Bast cells. Aggregate crystals.
39. Eupatorium.—Trichomes. Pollen grains.
40. Galla (Chinese).—Trichomes.
41. Gentian.—Fragments of large reticulate ducts.
42. Glycyrrhiza.—Groups of yellow tissue with crystal-bearing fibers. Cork tissue in unpeeled licorice. Fibrous tissue of trimmings.
43. Gossypium.—Bast. Fibrous tissue groups.
44. Haematoxylon.—Colored tissue. Reaction with copper solution.
45. Hamamelis.—Trichomes. Sclerenchyma cells.

46. Hyoscyamus.—Non-glandular trichomes.
47. Ipecac.—Raphides. Fibrous tissue. Starch.
48. Iris florentina.—Starch. Crystals.
49. Jalap.—Resin-bearing cells. Starch.
50. Kamala.—Glands and trichomes.
51. Krameria.—Bast cells and groups of bast.
52. Lobelia.—Trichomes and groups of fibrous tissue.
53. Lupulin.—Number of glandular structures.
54. Lycopodium.—Number of spores.
55. Mezereum.—Number of bast fragments.
56. Nux vomica.—Trichome fragments and groups of endosperm tissue.
57. Paracoto.—As for coto.
58. Physostigma.—Starch granules.
59. Phytolacca.—Number of raphides.
60. Pimenta.—Groups of sclerenchyma cells and trichomes.
61. Piper, black.—Groups of endosperm cells and of pericarp tissue.
62. Piper, white.—Groups of endosperm cells.
63. Prunus serotina.—Bast and sclerenchyma.
64. Prunus virginiana.—Bast.
65. Pyrethrum flowers.—Pollen grains. Sclerenchyma. Fibrous tissue.
66. Quillaja.—Bast and crystals.
67. Rhamnus purshiana.—Groups of bast and crystal-bearing fibers.
68. Rheum.—Aggregate crystals. Ducts. Colored tissue.
69. Sarsaparilla.—Number of raphides. Ducts. Starch.
70. Sassafras.—Bast. Groups of sclerenchyma cells.
71. Scopola.—As for belladonna leaf and root.
72. Senna.—Trichomes. Neighboring cells.
73. Stramonium.—Non-glandular trichomes.
74. Strophantus.—Trichomes.
75. Tabacum.—Glandular and non-glandular trichomes.
76. Thea.—Trichomes and sclerenchyma cells.
77. Viburnums.—Bast, and bast and sclerenchyma cells.
78. Zingiber.—Starch granules. Cork tissue.

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LINCOLN, NEBRASKA,
April 4th, 1920.

THE CHEMISTRY OF THE HEPTANE SOLUTION.

BY EDWARD KREMERS.

3. PURIFICATION OF HEPTANE AND ITS PHYSICAL CONSTANTS.

BY DR. C. L. SHERK.

(*Concluded from November Number, p. 1052.*)

II. PHYSICAL CONSTANTS OF PURIFIED HEPTANE.

1. Boiling Point.

For the final determination of the boiling point the constant pressure apparatus constructed by Professor Mathews[24] was used under the direction of Dr. A. E. Koenig.

The boiling point of water as registered by the thermometer employed is 99.96°. Accordingly there is positive correction to be made on all subsequent readings of 0.04°.