NOTES ON CHEMICAL TESTS OF THE UNITED STATES PHARMACOPŒIA.

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Changes in industrial conditions, improvements in manufacturing and analytical methods, and experience of many analysts with the tests of the U. S. P., 8th revision, since its publication in 1905, have brought to light various deficiencies in standards and methods. Some errors and inaccuracies have been carried over from one edition to another, merely because attention was not drawn to them, owing to a former general lack of interest in U. S. P. standards. The comments here presented are in part results and experiences of the writer and his co-workers, in part quotations from the published literature on the subject. Many observations in these notes will be found superfluous and obvious by those having ample chemical knowledge and practical experience, but it is hoped that they will be of some use to others having more limited experience in this branch of analysis. In many instances it will be found that the observations made coincide with those of others engaged in the same field, but it has not been found practicable to acknowledge priority in every case among a considerable number of subjects, as this would have required a complete search of the literature, which could not be done for lack of time. Any injustice that may have been done any one in this respect, is unintentional and, it is hoped, will be so regarded.

The writer takes pleasure in acknowledging his indebtedness to Dr. George D. Rosengarten for facilities kindly placed at his disposal and to Mr. Joseph Rosin, of this laboratory, for much of the information given in the following pages.

GENERAL METHODS.

SELECTIONS OF SAMPLES.—While directions concerning this subject may be beyond the scope of a Pharmacopœia, this matter is decidedly of practical importance in connection with the examination of medicinal substances. Disputes have often been caused by a lack of proper methods in sampling. The following is abstracted from *Lunge's Chemisch-Technische Untersuchungsmethoden,* 5th ed., vol. I, pp. 8-22: Selection of representative samples is most difficult with materials in a coarse state of division, when not homogeneous in composition. In such cases care must be exercised that a due proportion of fine and coarse particles is taken and that materials that readily lose or absorb moisture are not exposed to the air longer than necessary. It is often advisable to take large samples from different parts of a consignment, reduce the entire sample to a moderately fine state of division, mix well, and take a smaller sample from this for analysis. The containers should be of such material that changes in or contamination of the samples is prevented. They should have well-fitting stoppers. Substances affected by light should be placed in containers impervious to light. Glass of dark amber color is often sufficient, in other cases a covering of heavy dark paper is required in addition. Sampling of liquids is comparatively simple. Semi-solids, if not perfectly homogeneous, should be thoroughly stirred before a sample is taken. The analyst should mix thoroughly samples received of solid or semi-liquid materials before taking out any portion of them for analysis.

SPECIFIC GRAVITY.—The specific gravity data given in the U. S. P. are usually based on weights taken in air with brass weights. The data in some of the tables are corrected to a vacuum basis. The difference, however, is too small to be of importance in ordinary practical work. For liquids a pyknometer is always preferable when any degree of precision is required. For general laboratory use the Squibb pyknometer has been found more suitable than any other. Descriptions of it may be found in *Remington's Practice of Pharmacy* and in *Allen's Commercial Organic Analysis*. For an illustration of its use see SPECIAL PART, under *Aether*. The removable neck makes it eminently suitable for viscid liquids, as well. The average Mohr-Westphal balance is less accurate, not always trustworthy to the third decimal place, and the same may be said of most hydrometers.

SOLUBILITY.—Solubility determinations of solid chemical substances in various liquids are sometimes important aids in establishing purity, when other methods are insufficient, but it is well known that results vary greatly with the methods used. No instructions are given in the U. S. P. nor in most other pharmacopœias. The Swiss Pharmacopœia gives instructions as follows: The substance, in fine powder, is shaken from one-half to one hour at 40° to 50° with a quantity of the solvent insufficient for complete solution and the mixture then cooled to 15°, with constant shaking. The quantity of dissolved substance is determined in the clear liquid either by evaporating a weighed quantity and weighing the residue, by taking the specific gravity of the solution, or by other suitable analytical methods.—This method has the disadvantage that often a supersaturated solution is obtained unless allowed to stand at the standard temperature an unreasonably long time. Another source of error lies in the employment of an excess of the solute. If this contain a considerable amount of soluble impurities and a decided excess be taken, a disproportionate amount of the impurities may be dissolved and the solubility be thus found too great. The more accurate methods, however, require special apparatus not found in the average laboratory and too much time. For an approximate determination, exact enough for many purposes, the substance is passed through a silk sieve (about 50 meshes to the linear cm.), a weighed quantity is shaken with an insufficient amount of the solvent (measured or weighed) for a few hours, then small quantities of the solvent are added from time to time until solution is complete.—For an elaborate study of this subject by Atherton Seidell, the reader is referred to *Hygienic Laboratory Bulletin No. 67* (1910).

MELTING POINT.—No official method for melting point determinations is given in the U. S. P. That greatly divergent results are obtained by different methods is well known. For a detailed study of this subject by George A. Menge, see *Hygienic Laboratory Bulletin No. 70* (1910). The capillary tube method, as applied to organic and some inorganic chemicals in powder form, is too well known to call for a description here.—*Drying of Sample.* To prepare the substance to be tested, directions are frequently given,—e. g., by the German, Austrian, and Swiss Pharmacopœias, that the fine powder be dried uniformly for at least 24 hours in a desiccator containing sulphuric acid. This will cause discrepancies with substances containing crystal-water that do not yield their water readily at ordinary temperatures. With compounds containing no crystal-water the rate of desiccation varies greatly also; very hygroscopic compounds may require much longer than 24 hours, while others may be dried in this manner in a much shorter time. A better practice would be to continue the drying until a constant weight is reached. In laboratory practice a saving of time is often important and it is then preferable to dry the powder (sifted through silk having about 40 meshes to the linear cm., as advocated by Menge) at an elevated temperature, low enough to prevent other changes, such as fusion, partial volatilization, or decomposition. Compounds containing crystal-water in some cases can not be rendered anhydrous by heat without some decomposition, unless it be done in a vacuum dryer. As many anhydrous compounds absorb water from the air more rapidly than is usually appreciated, suitable precautions to prevent this absorption should, of course, be taken.—*Charging the Tube.* As a result of experiments, Menge recommends that the inside diameter of the capillary tubes be not less than 0.8 mm. and not more than 1.25 mm. The interior of them should be kept clean and dry, preferably by sealing both ends immediately after drawing them out and dividing them into the required lengths or by keeping the supply, open at one end, in a desicator. The charge of sample, when tapped down, should form a solid column in the bottom of the tube not less than 2.5 mm. nor more than 3.5 mm. high.—*Thermometers.* Menge recommends a set of three standardized thermometers, about 30 cm. long, ranging, respectively, from —10° to 150°, 100° to 250°, and 200° to 350°. Since all pharmacopœial substances, for which melting point determinations are of value, melt below 260°, a set of two or three, covering a total range of about—10° to 260°, would answer. The Anschuetz thermometers, sold in sets of seven or less, are still better adapted to this purpose, having the advantage of obviating the need of emergent stem corrections, when a suitable bath is used. They are 12.5 to 15 cm. long and each one covers a range of about 60°.—*Baths.* Immersion of the capillary tube and thermometer directly into a liquid bath is by many considered preferable to the use of an air-bath surrounded by a liquid. The latter, however, has decided advantages and if generally adopted as a standard method, would answer equally well for pharmacopœial tests, as regards accuracy. The objections raised against the air-bath, aside from an alleged tendency to give too high results, are the difficulty of construction by the analyst and the cost when purchased (the cost of a Roth apparatus is $1.80). An apparatus similar to that of Anschuetz and Schultz may be made from a small round-bottom flask having a rather long neck, and wide enough for insertion of a test-tube of 15 mm. diameter. The height of the test-tube is adjusted by means of a ring cut from thick rubber-tubing, which is fitted around the tube and rests upon the flared neck of the flask. The flask is two-thirds filled with sulphuric acid and rests upon wire-gauze. A long test-tube inverted over the stem of the thermometer makes emergent stem corrections unnecessary for low melting substances. An apparatus so constructed has been used with satisfactory results for years. Comparing the results obtained by using a Roth air-bath apparatus with those obtained by using liquid baths, upon 6 compounds, Menge found differences of from 0° to 0.6° only. Advantages that may be claimed for the air-bath are (1) that no fumes from a liquid bath can enter into the capillary tube and affect the melting point, (2) that no stirring is required, (3) that one outer bath, sulphuric acid, is sufficient for all purposes, (4) that no emergent stem corrections are needed when short thermometers are used.—Menge advocates for a bath a round-bottom, straight glass tube of 30 mm. internal diameter and about 100 mm. long, containing sulphuric acid for ordinary use or cottonseed oil for higher temperatures.—*Rate of Heating.* Menge recommends a uniform rate of heating of 3° per minute from 25° below the expected melting point until melting begins and a rate of 0.5° per minute during the melting interval. The importance of a uniform rate of heating cannot be overestimated, as no other factor influences the results so much as this.—*Reading of Melting Point.* The temperature at which the substance begins to melt should be recorded as well as that at which it is just completely melted, a narrow melting interval indicating a higher degree of purity than a wide interval.—*Correction.*. Corrections for the lower temperature of that portion of the mercury thread, which is above the bath, should always be made. The formula of Kopp, given below, indicates the number of degrees centigrade to be added to the observed melting point:

$$\text{Correction} = 0.000154\,N(T-t)$$

T = observed melting point; N = length of emergent thread of mercury above the bath in degrees Centigrade; t = mean temperature of emergent mercury-thread, measured by a small auxiliary thermometer the bulb of which is placed midway between the surface of the bath and the top of the mercury-thread of the main thermometer.—To illustrate the corrections required at different temperatures for thermometers of varying construction, Menge gives the following figures for two thermometers tested by him with use of a sulphuric acid bath, No. 1 being 300 mm. long and graduated from—6° to 406°, No. 2, about 310 mm. long and graduated from—10° to 263°.

Temperature of bath	80°	100°	120°	140°	160°	180°	200°
Correction for No. 1	0.43	0.54	0.90	1.38	1.97	2.63	3.39
Correction for No. 2	0.48	0.85	1.33	1.91	2.57	3.36	4.22

Melting Point of Fats.—The German Pharmacopœia gives the following directions: The melted fat is drawn into a tube of thin glass having an internal diameter of 0.5 to 1 mm., bent in U-form, in such manner that the fat forms an equally high column in each side of the tube. It is then kept for two hours on ice or for 24 hours at 10°. When attached to a suitable thermometer so that the fat is at the same height as the bulb, it is immersed into a test-tube of about 30 mm. diameter, containing a mixture of equal parts of glycerin and water. The upper open ends of the tube must be above the surface of the liquid. The bath is warmed very gradually. The temperature at which the fat becomes entirely clear is considered the melting point. The writer prefers this method with the addition that the bath is constantly stirred, that the warming is at the definite rate given above, and that the whole melting interval is recorded.

CONGEALING POINT.—No directions for the determination are given in the U. S. P. The German Pharmacopœia directs as follows: About 10 gm. of the substance to be tested are melted in a test-tube of 20 mm. diameter. By immersion in water, the temperature of which should be about 5° lower than the expected congealing point, the liquid is cooled to about 2° below the congealing point, then stirred with the thermometer, with the addition of a small crystal of the same substance if necessary, to induce crystallization. The highest temperature recorded during the solidification is recorded as the congealing point.—If the congealing temperature differs much from that of the room, influence of the latter is lessened by placing the tube containing the liquid within a larger tube, to serve as an air-bath, as the change of temperature during the congealing interval should be very gradual, as in the case of melting point determinations.

BOILING POINT.—For purposes of identification, the following method is given in *Mulliken's Identification of Organic Compounds:* One or two drops of the liquid are placed in a thin glass tube, closed at one end, about 3 mm. in diameter. To regulate ebullition, a small capillary tube, open at the lower end, but closed about 2 mm. above the lower end by fusing the walls together, is inserted into the larger tube, which is then attached to a thermometer and heated in an apparatus such as is used for melting point determinations. The temperature at which an uninterrupted series of bubbles begins to rise is considered the boiling point.—For a test of purity at least 50 cc. should be distilled from a flask having about 50 per cent. greater capacity and provided with a side exit-tube. To regulate ebullition, fragments of pumice stone or platinum scrap should be added. The upper end of the thermometer-bulb should be slightly lower than the exit-tube. For low temperatures water-baths or air-baths are suitable, for high temperatures sand-baths or wire-gauze. To prevent overheating the vapors of low boiling substances by radiation from the bath, a circular sheet of thick asbestos, about 20 cm. in diameter and having a central circular opening about 3 cm. in diameter, should cover the bath before the flask is placed upon it. Distillation, when about 50 cc. are taken, should be at the rate of about one drop per second.—The boiling points of the U. S. P. are in greater part taken from sources that include corrections for the emergent mercury thread and for varying barometric pressure. These should, therefore, not be omitted. The first is made in the same way as described under *Melting Point*. Employment of short thermometers of the Anschuetz type make the correction unnecessary. Extreme variations of atmospheric pressure may raise or depress boiling points to the extent of 1° or more. According to a formula of Kopp, a correction of 0.0375° for each mm. pressure differing from 760 mm. is required, to be added to the observed boiling point when below or substracted when above. This correction applies to variations of 720 to 780 mm. only.

TESTS OF IDENTITY AND PURITY.—In a number of instances the U. S. P. makes statements that may leave the analyst in doubt as to whether they are intended merely as information or as definite requirements. This applies particularly to physical constants, which are sometimes given in great abundance, when one or two of them would be sufficient to determine the quality of a substance. It is desirable that, when these are considered necessary for establishing identity or purity, the wording be such, that no doubt need be felt as to the exact requirements. Too much is at present left to the discretion of the analyst, who may contend that only such tests need be made as are distinguished by such words as "should" or "must." The statement often made in parenthesis after tests that failure to respond to reactions for impurities tested for implies "absence" of them, in many cases is not strictly accurate, as the tests are frequently not sensitive enough to detect minute traces. There is at least one instance on record that a buyer demanded complete absence of certain impurities on the authority of these statements, which was equivalent to demanding a chemically pure product. It seems preferable, in view of this, that only the names of the impurities and adulterants be given after the tests. Another source of controversy is a difference in interpretation of such requirements as "no turbidity should be produced on the addition of barium chloride T. S.," without mention of a time-limit. It may be contended that no visible reaction should take place after an indefinite period, while a more reasonable interpretation would be that the requirement is met when no reaction takes place at once or within a few minutes. A definition of the exact meaning to be conveyed by such statements as "a solution of potassium hydroxide (1 in 4)" or "diluted sulphuric acid (1 in 2)" would be desirable. It will obviously make some difference in the strength of the diluted acid, whether 1 cc. or 1 gm. of the acid (sp. gr. 1.84) be diluted, also, whether the dilution be made *with* 2 cc. of the diluent or dilution be made *to* 2 cc. It will be seen that four interpretations are possible. While in most cases, when dilution is greater, the difference is negligible, in some instances it may exert an influence on the results of tests. In absence of a definition it seems permissible to assume that 1 cc. of concentrated sulphuric acid is to be diluted with enough of the diluent to make 2 cc. and that 1 gm. of potassium hydroxide of U. S. P. strength (85 per cent.) is to be dissolved in enough of the solvent to make 4 cc. A general rule is desirable as to the quantity of solution to be taken for a test involving turbidity, color and odor reactions, etc. For instance, a slight opalescence visible in a bulk of 20 cc. may be invisible in 10 cc. or 5 cc. of the same solution; it may be visible in a tube of 30 mm. diameter, but invisible in one of 10 mm. diameter, when viewed transversely. It is to be inferred from specific directions given in some cases, that about 10 cc. should be taken and the test made in a tube of about 20 mm. diameter, unless otherwise stated. The quantity of reagent might also be given to advantage, as the delicacy of a test often depends on this. The Netherlands Pharmacopœia directs addition of 3 drops of the test solution to 5 cc. of the solution to be tested for such impurities as chlorides, sulphates, calcium, iron, etc.—*Time Limit Tests for Heavy Metals.* It would seem that a specification of the internal diameter of the test-tubes to be used is more important than their capacity, which is given as 40 cc. A diameter of 18 to 20 mm. was probably intended, as that corresponds with the average dimensions of test-tubes of this capacity, but tubes of quite different dimensions are often used. The time-limit can be shortened to 15 minutes, as no increase in color or turbidity is ever noticeable after this interval.—*Modified Gutzeit's Test for Arsenic.* The hydrochloric acid used for this test should be practically free from arsenic. An acid of U. S. P. standard of purity, which is directed to be taken, may, and usually does, contain too much. It is better, however, to use sulphuric acid, as this is more readily obtained free from arsenic. So-called "shot" or "tear" zinc has been found preferable to the "mossy" form, as the evolution of hydrogen from it is more regular, the mossy kind often reacting too violently at the beginning. On the other hand, very pure shot zinc may react too slowly, but the action is readily accelerated by the well known expedient of adding a drop of a weak solution of platinum chloride or copper sulphate. Absorbent cotton has been used to advantage in place of cheese-cloth. It is difficult so to roll the cloth that it will be free from channels that will allow hydrogen sulphide to pass through. It has been found insufficient in many cases to evaporate with sulphuric and sulphurous acids merely to 5 cc. or until the odor of sulphurous acid is no longer recognizable. To insure complete removal of the latter, it is better to evaporate until no further reduction in volume takes place. The quantities of reagents directed for the blank test are much too small. Made according to present directions it fails to show the presence of such quantity of arsenic in the reagents as would be enough to vitiate the results of the tests made with them. A uniform limitation of 10 parts of arsenic per million appears hardly consistent in view of large differences in doses.

QUANTITATIVE METHODS.—A statement regarding permissible water contents in chemical substances, which has an important bearing on the purity "rubrics," is often overlooked because of its inconspicuous place in the Preface of the U. S. P. (p. xxxviii). Many compounds, when "sensibly" dry, may fail to meet the rubric requirements fully, unless allowance be made for adhering moisture or for chemically combined water in hygroscopic "exsiccated" salts, which is permitted in the statement referred to.—*Determination of Water.* For the determination of crystal-water or adhering moisture the sample should always be placed in a container provided with a well-fitting cover, as most anhydrous compounds absorb water from the air with more or less avidity. Anhydrous quinine sulphate, for example, may easily increase 1 per cent. in weight, while it is weighed in an open dish. For light, bulky substances it has been found concenient to use shallow dishes of thin glass, with straight, vertical sides and flat bottom, about 5 cm. in diameter and 2.5 cm. high, and with ground-glass covers, fitted in the manner of the stoppers of ordinary weighing bottles. For ordinary purposes the customary "watch-glasses" with ground edges, in pairs, and held together with metal clamps, answer as well.—*Weighing.* While the weighing of quantities in round numbers or of quantities having some relation to molecular weights, as it is often directed in the U. S. P., has certain conveniences in simplifying calculation of results, this plan is usually open to grave criticism. With liquids such a course is entirely out of the question and with solids it is excusable only in rare cases, when the necessary exposure to air during the weighing is known to be totally without influence on the substance. Even then the highest degree of accuracy in weighing cannot be attained by this method. Hygroscopic and distinctly efflorescent materials cannot be weighed in this manner with even a moderate degree of accuracy. Only glass-stoppered weighing-bottles should be used for this purpose, *about* the desired quantities placed into them and weighed in the stoppered bottles. Fuming acids and other volatile liquids are best weighed in bottles containing enough of the diluent or solvent to be used in the analysis to prevent loss. When heat is generated by such admixture, time must be allowed for cooling to room temperature and air admitted to the bottle, after cooling, to equalize the pressure, before weighing. Residues obtained after heating in a crucible should always be weighed in the *covered* crucible, with all avoidance of needless exposure to the air of even the covered crucible before and during the weighing, unless the substance is known to be non-hygroscopic.—*Volumetric Analysis.* It is often advisable for accurate titrations to take larger quantities of the sample than given in the text of the U. S. P. When an error of 0.1 or 0.2 per cent. is of importance, enough should be taken to require nearly a full burette of the volumetric solution, but not so much that the burette has to be filled more than once, as this would double the error of reading the volume. The best burettes for precise work have an internal diameter of about 10 mm. and are calibrated with ring-like markings instead of short lines. These rings are aids in bringing the eye to an exact level with the meniscus. When volumetric solutions are used at temperatures differing considerably from that at which they have been standardized, which is sometimes unavoidable, a correction for change in volume is required. N/1 solutions require a correction of about 0.1 cc. for each 100 cc. used, for a variation of 5° from the standard temperature, to be added when below, subtracted when above, N/5 and weaker solutions require a correction of about 0.5 cc. under the same conditions (see *Lunge's Chem.-Tech. Unters.-Meth.*, 5th Ed., V. 1, p. 55). Solvents and diluents intended for acid and alkali titrations should be neutralized before using, as they are seldom perfectly neutral. The same indicator should be used that is to be taken for the titration. Doubtless through an oversight, the U. S. P. directs titration of organic acids with alkali solutions that have been standardized at a boiling temperature, without stating that a boiling temperature should be maintained during the titrations, except in the case of lactic acid. Moreover, heat cannot be employed in titrating volatile acids. For such purposes, therefore, the alkali solutions should be standardized cold, which is readily done with potassium bitartrate that has been passed through a fine silk sieve before drying. The official methods for standardizing acid and alkali solutions are defective in that solutions standardized with one indicator are used for titrations with another indicator. As has been shown by Treadwell, methyl orange is not entirely insensitive to carbon dioxide. The discrepancy caused by a change of indicators is greater than can be ignored for precise work. The writer favors standardization with potassium bitartrate for phenolphthalein titrations and anhydrous sodium carbonate, made by heating sodium bicarbonate at 260° to 280°, for methyl orange. It has been pointed out by Treadwell, Lunge, and others, that sodium thiosulphate, when dissolved in water containing carbon dioxide, continues to deposit sulphur for some days. To the directions of the U. S. P., therefore, should be added, that, unless freshly boiled water is used, the solution should be allowed to stand a week before it is standardized.—*Non-volatile and Non-combustible Residues.* The U. S. P. frequently states of combustible or volatile substances, that they are "completely" volatilized by heat or that no residue should remain after combustion or that they should leave no weighable residue, when the quantity of material to be taken is not mentioned. These statements cannot be taken literally, since weighable residues can always be obtained when a sufficiently large quantity of the sample is employed. It is now possible, as a result of a large number of quantitative determinations of such residues, to suggest practical, allowable limits. The meaning of the term "unweighable" is not defined in the U. S. P.

The German, Netherlands, and Belgian Pharmacopœias define quantities less than 1 mg. as unweighable, the Swiss and Danish Pharmacopœias quantities of 0.5 mg. and less. Crucibles or dishes of platinum are usually preferable to porcelain for exact determinations of non-volatile and non-combustible matter, on account of better heat conduction and because of reaction of some ash constituents with silicates at high temperatures, with a consequent loss in weight of the residues. For quantities of 0.1 to 0.2 gm., platinum crucible lids are generally best suited. The German Pharmacopœia give the following directions for ash determinations: A suitable quantity is first carbonized in a crucible at a low temperature, then incinerated. To hasten combustion of the carbon, the flame is removed from the crucible occasionally for a short time. If complete incineration cannot be attained by a moderate heat, the mass is mixed with hot water and transferred to a filter of known ash content. The filter and contents are washed with a little water, then returned to the crucible, dried and incinerated. After cooling, the filtrate and washings are added and evaporated on a water-bath after addition of a little ammonium carbonate. The residue is heated to dull redness, cooled, weighed, and filter ash subtracted.

(To be continued.)

STATE BOARD OF PHARMACY QUESTIONS ON CHEMISTRY.

A. H. CLARK.

At the joint session of the Section on Education and Legislation with the Boards of Pharmacy, and Pharmaceutical Faculties, held during the Boston meeting and reported in the Bulletin of the American Pharmaceutical Association for December, 1911, there was presented the report of the Committee on Examination Questions. This committe was appointed by the Chairman of the Section in accord with a resolution passed during the Richmond meeting (1910).

I take it that the sole object of this work is to bring about a discussion of the subjects which are of mutual interest to the Boards of Pharmacy, Pharmaceutical Faculties, and to the candidate before these Boards and Faculties, as well as to the pharmacist in general. Free discussion and liberal criticism are the best means of furthering this end, and as I had not the pleasure of attending this particular session, I am taking the liberty of presenting here a few remarks on the questions submitted as proper for candidates writing on chemistry.

Examining bodies such as Boards of Pharmacy, must bear in mind that the candidate who appears before them may have secured his knowledge of chemistry from any of the following sources: He may be a university graduate, he may be a graduate from a college or school of pharmacy, he may have obtained his knowledge from some correspondence course, he may have obtained it from some night school, or from some "quiz course," or from a private tutor, or from his employer, or by individual effort alone. These conditions are made possible by the variation in requirements exacted by the different State Pharmacy Laws. For this reason no one set of questions can be prepared to fit every case, and each