The Ultra-Microscope*

And Its Application to the Study of Colloids

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It is often imagined, by those unacquainted with either of the sciences of light and microscopy, that it is merely a question of difficulties in construction of the microscope, particularly of the lenses employed, which prevents us from perceiving the very smallest of objects by its aid. Such, however, is not the case. At certain definite limits of size, phenomena intervene which effectually mar every effort at the microscoping of objects with diameter below this limit. The root of the difficulty lies in the phenomenon of light itself. The fact that light is

Fig. 1.—General Arrangement of the Optical System for Illuminating the Field of the Ultra-microscope.

a transverse vibration of appreciable wave length is of slight importance when dealing with the common objects of everyday experience, but immediately the attention is turned to objects of size comparable with this wave length, the mechanism of light propagation assumes tremendous significance. Then it is that the ordinary laws of reflection and refraction are waived in favor of the more subtle, and therefore the more interesting phenomena of diffraction.

On the common sense principle that a sea wave is but little disturbed by a pebble, though profoundly modified when brought up against a sea wall, it seems reasonable to assert, without further explanation, that an object comparable in size with a wave length of light cannot be expected to reflect waves which could convey to the eye a distinct impression of its size and shape. True, it does effect a minute disturbance, but of this more anon. Without venturing into a mathematical discussion of a microscope's "resolving power," it will be sufficient to state that one cannot hope to perceive an object whose size lies below one quarter of a wave length of light, this constituting the limit above referred to.

However, fortunately or unfortunately, scientists have interest in objects much smaller than these. Thus, the ever-increasing subject of colloids demanded an instrument for the direct observation of colloidal particles; and bacteriology, again, found itself hampered for want of a means of visualizing the cells and microbes with which it is concerned. The difficulty has been happily surmounted by the introduction of the ultra-microscope, an instrument which, as its name implies, renders it possible to demonstrate to the eye the existence of objects invisible ordinarily to the finest microscope.

This does not imply that the ideas put forward as to the limits of optical resolution have been in the least contradicted, as the following consideration will show. To be seen, an object must either reflect the light by which we see it or be self-luminous. Consider only a very small particle. If it be of a magnitude above the optical limit, it may be seen by virtue of the light it reflects, and its true form can then be perceived. If, however, its magnitude be below this limit, reflection is impossible, and all incident light becomes diffracted, i. e., scattered in all directions. In the latter case, the object emits light exactly as though it were luminous of itself, though under ordinary circumstances it remains invisible because the proportion of light reaching the eye with regard to the incident light is so small. If now means be adopted for concentrating light upon the object, it might be possible, other circumstances being favorable, to make it emit sufficient light to reach visibility. Of course, the object then cannot be seen in the sense that its shape or surface can be observed, but only seen in the sense that its presence is indicated by the light it emits, being observed in the microscope as a minute disk of light. The utilization of such a concentrated illumination is the fundamental idea underlying the construction of the ultra-microscope.

This principle was foreshadowed by a phenomenon observed long ago by Tyndall, who passed an intense beam of light through the air of an otherwise darkened chamber, when the track of light was made clearly visible by the light diffracted from the minute dust-motes floating in the atmosphere. Ultra-microscopy furnishes an extension of this, a microscope being introduced for the fuller examination of the track of light. Thus, Zsig-

mondy, in 1900, as part of his observations on some colloidal gold solutions, reflected a cone of light into the solution and examined the apex of the cone through a microscope. Working on the same principle, Siedentopf was led in 1903 to the improvement of the apparatus, with the result that we owe to him the introduction of the modern high-power illuminating arrangements which form the main feature of the instrument.

Naturally, certain conditions require to be fulfilled. In the first place, the most intense illumination possible

is necessary to render the smaller particles visible; secondly, to prevent dazzling of the eye and consequent inability to detect the minute light disks, no illuminating ray must be allowed to fall upon the eye either directly or by reflection; thirdly, in view of the often extreme faintness of the light disks, the darkest possible background is essential; and lastly, the beam of light must be extremely shallow in the direction of the line of sight, else nothing more definite would be observed than a luminous haze. Only by rigid satisfaction of these conditions can definite results be obtained.

In the light of the above requirements, the following diagrammatic arrangement of the apparatus constituting

Fig. 2.—Cell for Examining Liquids.

one of the most delicate forms of the ultra-microscope and seen in Fig. 1 will be considered. The solar rays are reflected from a heliostat through the slit A into the darkened laboratory in which the apparatus is set up. The light falls on to B, a telescope objective, by which it is concentrated on to the slit C, which is capable of very fine adjustment. D is a screen with an aperture of sufficient size to cut off any stray rays of light reflected from the edges of the slit. Another objective, E, directs the light into the condenser F, from which it passes as an intense beam into the solution contained in G. At right angles to the path of light is arranged the microscope H for minute examination of the track of light in G. This

Fig. 3.—Illumination by the Method of Cotton and Mouton.

latter cell, for the use of solutions, usually takes the form shown in Fig. 2, the rectangular part K having quartz faces and being fitted also with a funnel and outlet, which permit of easy washing and filling without disturbing the adjustment.

The above design by Siedentopf of the ultra-microscope is not the only one which has been used. A simpler device, due to Cotton and Mouton, is worthy of note. An oblique parallelepiped of glass, surmounted by the solution and cover slip, is placed upon the stand of the microscope. The convergent illuminating beam follows the course shown in Fig. 3, the angle of incidence on the upper surface being adjusted to lie between the critical angles for water-glass and glass-air surfaces and the beam, therefore, is totally reflected. Then, the only light which enters the microscope H is that diffracted by the ultra-microscopic particles of the solution.

For the examination of larger particles, e. g., cells, bacteria, and so on, modified forms of the apparatus may be employed, in which, for convenience, the illuminating rays and the rays diffracted from the particles are in the same straight line. But as these would require complicated designs and descriptions, their consideration is omitted.

To understand at all completely the beauty of this apparatus, resort must be made to a consideration of several of its more interesting applications. Its possible service to the colloidal chemist and to the bacteriologist has already been hinted at, and we cannot do better than turn our attention to at least one of these applications. Before so doing, a few figures indicating the degree of sensitiveness as compared with other methods of analysis might be deemed interesting. By the aid of spectrum analysis, Bunsen and Kirchhoff consider that 0.14×10^{-6} milligramme of sodium can be detected; while, in the case of hydrogen, Emich avers that as minute a quantity as 0.7×10^{-13} milligramme gives appreciable indication of its presence. Again, it is asserted by Fischer that by the sense of smell 2.2×10^{-9} milligramme of mercaptan and by Berthelot that 10^{-11} milligramme of iodoform are capable of detection, though here the examples are far and away among the most exceptional that could have been chosen. In the chemical way, too, 3×10^{-7} milligramme of sodium hydroxide are able to produce a definite change in the color of some suitable indicator. With the ultra-microscope, however, a particle of gold of mass 10^{-16} milligramme in a gold ruby glass may be observed without trouble, while when the most favorable conditions are available, a particle of size ten times that of an average chemical molecule will not escape observation. Obviously, the potentialities of the instrument are great and there is no saying what more astounding results may be expected when the method is still further improved.

APPLICATION TO THE STUDY OF COLLOIDS.

No more fascinating field of inquiry is open to science than the investigation of so-called colloidal solutions. That such a substance as platinum, for instance, generally regarded as insoluble in water, should be capable of forming what appears to be a solution, is certainly striking on first acquaintance. Not only metals, however, but many "insoluble" substances, like silver chloride, can be made to develop this peculiar state by suitable methods. Natural colloids are of universal distribution, the very constituents of living cells appearing to exist in such a form. The properties of a colloidal solution afford a striking contrast to those of ordinary solutions; for instance, their slight ability to diffuse and consequent separation from truly soluble salts by dialysis, their insignificant osmotic pressure, their peculiar electrical properties which seem to point to the presence of an electric charge upon the particles, their capacity for coagulation and absorption, and so on. All these circumstances add an interest to the study of colloids which has served to attract a great host of investigators, as will be gathered from the enormous output of work upon the subject.

Much controversy has been waged over the exact nature of these colloidal solutions, for they bear intimately upon the problem of solution in general. Many investigators have been inclined to the idea that they represent perfect solutions, while others identify them as suspensions. The intervention of the ultra-microscope has contributed largely to the development of the question, if not to its final solution.

With one or two exceptions, all colloidal solutions on ultra-microscopic examination are found to contain distinct particles, which reveal themselves, as already stated, in diffraction-patches on a grey background. These particles can be counted and their size calculated. There is thus strong evidence for regarding a colloidal solution as merely a limiting case of a suspension. A crystalloidal solution, i. e., the solution of a substance like sodium chloride, gives an absolutely clear field, and one, therefore, infers the absence of even the tiniest particles in a solution of this type. Such a conclusion, however, is questioned by the observations of van Calcar and de Bruyn, who found, for example, that rapid centrifugalization of a sodium sulphate solution induced partial separation of the salt. From the above reasoning, and further observation but confirms this, it seems impossible to draw any sharp line of demarcation between suspensions, colloidal and crystalloidal solutions, the one class merging imperceptibly into the other. Ultra-microscopic study shows over how wide a range the size of the particles in a colloidal solution varies and renders any attempt at classification of solutions still more un-

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satisfactory. Indeed, it seems probable that in a solution the size of the "dissolved" particle can vary gradually from that of the ordinary chemical molecule to the dimensions of a visible suspensoid particle, the properties of the resulting solutions varying with the molecular forces called into play.

But more interesting developments even than these can be attributed to the ultra-microscope, for results recently obtained by its aid go far toward strengthening the probability of the Atomic and Molecular Theories. Over a century ago, the naturalist Brown observed that the particles of a fine suspension, when observed under the microscope, are in a continual state of agitation, but the observation was shelved and almost forgotten. It is only in recent years, and mainly by the application of the ultra-microscope, that the further investigation of this interesting phenomenon has been rendered possible.

The ultra-microscope has demonstrated that the smaller the particle the greater the activity, until in colloidal solutions we get a movement so violent as to resemble, in the words of Zsigmondy, "a swarm of dancing gnats." There seems to be some connection between this rapid movement of small bodies and the slower movement of the heavier particles observed by Brown. Indeed, it almost suggests that, if further diminution of the particles be imagined, the attainment of molecular dimensions might give a value for the molecular velocity of an order comparable with that calculated on the theoretical assumptions of the Kinetic Theory; in other words, that the kinetic energies of a molecule and of a colloid or suspensoid particle are equal. This view has developed almost into a certainty, particularly by Perrin's remarkable investigations, and an experimental verification of the Kinetic Theory is thus forthcoming.

A brief outline of Perrin's work will serve not only as a development of the above brilliant idea, but also as a

fitting demonstration of the methods employed in ultra-microscopic research.

In the first place, his investigations required a colloidal solution, the particles of which were of the same size, and this was rendered possible by the method of "fractional" centrifugalization. The uniform solution, after dilution and standing for some time, was then examined ultra-microscopically, the microscope being focussed on an extremely shallow beam of light capable of vertical movement. At positions corresponding to different heights of the solution, an estimate of the number of particles was accurately made. This, of itself, is an extremely laborious undertaking. The particles illuminated by the beam are visible in the field of the microscope, but are executing their Brownian dance, and it is impossible to make even an approximate computation of their number. The difficulty is overcome by the use of one of two methods. Either the field of view is instantaneously photographed several times and the mean number taken; or a stop is inserted in the microscope so as to limit the field to contain only a few particles, and then, by means of a shutter, instantaneous "peeps" are obtained at small intervals, the number of observed particles being noted at a glance, and subsequently the mean of several thousand of these readings taken. Both methods give very concordant results. Having ascertained the number of particles corresponding to different heights in the solution, examination showed that these were, within the limits of experimental accuracy, exactly in geometrical progression.

Thus, at heights 100 75 50 25 microns,
the numbers 200 170 146 116 were obtained,
while 201 169 142 119 are in exact geometrical progression. Such results demonstrated beyond doubt that the particles reach a state of equilibrium where their distribution corresponds to that of the mole-

cules of a fluid. Thus, in the atmosphere, the concentration of the constituents gradually diminishes as we ascend, the diminution obeying the above exponential law. The only difference between the two cases is that while the atmosphere requires a rise of six kilometers for the halving of the concentration, a colloidal solution requires about one tenth of a millimeter.

Having proved that such an experimental law was applicable to colloidal solutions, a mathematical expression was easily deduced by which, given the density of a particle, its mass and the number per unit volume, the Kinetic Theory constant—which denotes the number of molecules per gramme-molecule of any compound—is calculable. For the present purpose it is not necessary to go into the mathematics of the case. Sufficient that the mass is now the only unknown which prevents us from solving the problem, and this is deduced by the application of Stoke's law, which gives an expression for the velocity of a sphere as it sinks through a viscous fluid. The velocity of fall of colloidal particles in a capillary tube, as they descended to take up their final distribution, was carefully observed and by substitution in Stoke's equation the mass of the particles calculated.

Everything is now ready for the determination of the Kinetic Theory constant. On the basis of this theory, the constant has been worked out to be 7×10^{-23} . Judge of the interest and importance of the above investigation, absolutely experimental throughout, when the value of the constant was found to be 6.9×10^{-23} . Since then, more accurate determinations have been made and it seems that the above method comprises a means for the determination of that fundamental constant, the number of molecules per gramme-molecule, which is capable of almost unlimited precision. The gas laws, already applied by van't Hoff to dilute solutions, are thus extended by Perrin to uniform emulsions.

The Nitrogen Enrichment of Soils*

In a paper recently read before the Royal Society of Canada the authors gave a summarized account of the experiments that had been conducted by the Chemical Division of the Experimental Farms since 1889 in the very important matter of the nitrogen enrichment of soils ("Experimental Work towards the Nitrogen Enrichment of Soils," by Frank T. Shutt, and A. T. Charron.

The first fact brought forward was that rich productive soils are characterized by a high nitrogen and humus content. Evidence was adduced to show the correctness of this statement, not only from the examination of cultivated soils of first quality and established high productivity, but from many analyses of soils of the virgin prairie of the North-Western provinces so widely recognized for their wonderful fertility.

On the other hand, soils naturally poor and those impoverished by irrational farming have been found to be low in nitrogen and humus forming material. The semi-decomposed vegetable matter of the soil is the natural storehouse of its nitrogen, and experimental proof had been obtained that demonstrated beyond question that humus forming material must be constantly added to cultivated soils if their nitrogen content and their productivity were to be maintained. The grain growing of the West, which implies fallowing and no formation of a sod, was very destructive of humus and nitrogen, and must in time seriously impair the richest soil. The rational and economic upkeep of soil fertility demands the keeping of stock for the production of manure—the most important natural source of humus and nitrogen for farming lands—and a proper rotation of crops; that is, one which will periodically enrich the soil, as by the growth of a legume, in these valuable constituents.

Nitrogen is the dominant element among those furnished by the soil, and its amount and availability in a very large measure determine crop yield. The investigations discussed in this paper were instituted to learn the extent of the depletion of the soil's nitrogen by cropping and cultural operations, how far the legumes (clover, alfalfa, vetch, etc.) could be utilized on the farm for the increase of the soil's nitrogen and the furnishing of humus forming material, and finally to ascertain what the practical value might be of inoculation (a) with "cultures," and (b) with soil from fields bearing a leguminous crop for the encouragement of the growth of these plants.

DEPLETION OF SOIL NITROGEN.

It was found from the examination of a soil which had never received manure and which had been under cultivation for twenty-two years, during which period it had borne six crops of wheat, four of barley, and five of oats, with nine summer fallows (bare) during the latter seventeen seasons, that the loss of nitrogen to a depth of 8 inches amounted to 2,206 pounds per acre. Of this, approximately 700 pounds had been removed in crops. This means that about 1,500 pounds per acre, or 68 per cent of the total nitrogen lost, had been dissipated during this period through cultural operations, fallowing, etc.

* A contribution from the Chemical Division, Dominion Expt. Farms, Canada, published in the *Chemical News*.

The soil experimented with was an exceedingly rich prairie soil in Saskatchewan. Very probably it is a type of soil that would at first lose nitrogen more rapidly than one of a poorer quality, and, further, undoubtedly, fallowing is of all operations the most wasteful of soil fertility, but the figures are significant in showing there is an inevitable and heavy depletion of the soil's most valuable constituent, consequent upon the necessary tillage of the land. Our soils, then, must be constantly replenished with organic matter if they are to be kept productive and profitable.

LEGUMES AS NITROGEN ENRICHERS.

As is well known the legumes are very rich in nitrogen, and as a part of this nitrogen at least is obtained from the atmosphere, they as a class are extremely valuable as manurial agents. The more important leguminous crops, clovers, alfalfa, vetches, peas, beans, etc., were grown and analyzed, the weight and nitrogen content of stem, leaves, and roots per acre being determined. The clovers and alfalfa were found to be the most valuable, chiefly by reason of their larger root system, in which might be stored from one third to one half the total nitrogen in the crop. By the turning under of a fair growth of one of these crops, from 100 to 150 pounds of nitrogen may be added to the soil per acre, an amount equivalent to that furnished by an application of 10 tons of ordinary barnyard manure.

The details of an interesting and valuable experiment were given, in which by analysis of the soil "before and after" the amount of nitrogen which had become part and parcel of the soil through the growth of clover was determined. A plot of very light sandy loam was first seeded with clover in 1902. Every second year from that date until the present time the plot had been dug over and re-sown. No manure at any time was used, but phosphoric acid and potash were furnished at the outset by a moderate dressing of superphosphate and muriate of potash. The soil was sampled and analyzed six times during the experiment period. The data show that the nitrogen content had practically doubled in the nine years despite the losses from bacterial activity and other causes. The soil to a depth of 4 inches contained at the beginning of the experiment (1902) 533 pounds, and at the close (1911) 1,005 pounds per acre. If it is assumed that the growth of the clover had added annually nitrogen at the rate of 100 pounds per acre it will be observed that the loss due to oxidation, etc., during the experiment period almost equalled the gain.

If the clover had been cut and fed, the manurial value of the residues (roots, decayed leaves, etc.) would have been almost one half that here recorded. These data afford satisfactory evidence as to the value of a leguminous crop in the rotation, if soil fertility is to be economically maintained.

INOCULATION EXPERIMENTS.

The special value of the legumes as nitrogen enrichers is due to the fact that they are able to draw upon the free nitrogen of the atmosphere for a part of their supply. Thus, instead of impoverishing the soil's store like all other crops, they add to it. The appropriation of free nitrogen does not take place directly, but is accomplished

through the agency of certain bacteria present in the soil, which attach themselves to the roots of the legume, with the result that nodules or tubercles are formed thereon in which they reside. In some way, not as yet clearly understood, nitrogen compounds are formed within these nodules, and enter into the circulation of the host plant, to be built up into its tissues of root, stem, and leaf. It is the nitrogen of the air existing in the interstices of the soil that these special nitrogen-fixing micro-organisms utilize, and this points to the desirability of a well drained, well aerated soil if these bacteria are to perform their beneficial function. Without the aid of these specific bacteria the legumes cannot avail themselves of the free nitrogen of the air, but like other crops draw upon the nitrates of the soil for their nitrogenous food. Legumes, therefore, in the absence of these germs are not nitrogen enrichers of the soil. Are these nitrogen-fixing bacteria universally present in the arable soils of the Dominion? We cannot say that they are, though the examination of the roots of legumes collected in various parts of Canada has shown by the nodules thereon that these micro-organisms are very widely distributed over the Dominion. Our observations have made very clear that failure to obtain a good catch and growth of clover is not always to be attributed to the absence of the necessary bacteria, but in many instances is due rather to an unfavorable mechanical condition of the soil, inadequate drainage, deficiency of moisture, acidity or sourness of the soil denoting the need of lime, or finally to poor seed.

To furnish the farmers with the means of introducing the nitrogen-fixing bacteria, where their absence is suspected, "cultures" of these bacteria have been prepared and put on the market. Their use is known as inoculation. During the last twenty years a considerable number of these cultures or preparations, from Germany, United States, and home sources, have been tried on the Experimental Farms, and at other points. Specific cultures for clover, alfalfa, peas, beans, etc., have been experimented with. While in many instances they distinctly favored the growth of the legume, their action on the whole was more or less uncertain. The profitable employment of these preparations seems therefore problematical. All the failures met with were not attributed to lack of vitality in the cultures, but it was evident that their usefulness is much impaired by age, light, and heat, and unless prepared by a reputable firm or institution, and still fresh, good results can scarcely be looked for.

The employment, as an inoculating material, of the soil from the surface of a field bearing a luxuriant crop of the specific legume had given much better results than the use of cultures, and this method, where cost of transportation is not too great, will be found the most reliable for the general farmer. Notable instances of successful inoculation by this method, applying the soil at the rate of 100 to 300 pounds per acre, were recorded for alfalfa in the North-Western provinces. Inoculation, as has been said, is not generally necessary, but where it is considered indispensable by reason of the absence of the nitrogen assimilating bacteria, a supply of this bacteria-laden soil will, it is believed, prove more effective than the use of a culture.