Biography of Mikhail Semenovich Tswett and Translation of Tswett's Preliminary Communication on a New Category of Adsorption Phenomena

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Mikhail Semenovich Tswett
(May 14, 1872–June 26, 1919)

"Scientists are in fact dreamers and artists; they are not free with their ideas; they can work well and hard only at what their thinking accepts and what their feelings are drawn to. Ideas alternate: impossible and often mad ones appear; they swarm and whirl, fuse and sparkle. Scientists live among these ideas and work for them."

——Vladimir I. Vernadsky (1863–1945)
——Russian natural scientist, eminent thinker

In the twentieth century society made a huge leap in the development of science and industry. The creation of new materials and new branches of industry was inseparably linked with and, to a great extent, determined by the elaboration of new efficient methods in analytical chemistry. Achievements in exploring space and in creating new branches of industry (atomic, microbiological, macromolecular, and so on) as well as many discoveries in biochemistry and medicine would have been impossible without the elaboration and formation of distinctly new methods of analyzing substances. These methods, characterized by extremely high sensitivity and selectivity, were introduced into analytical practice. A chromatographic analysis of complex mixtures discovered by Mikhail Semenovich Tswett was one of the most important achievements in science and analytical methods in the twentieth century.

In 1896 M. S. Tswett presented his doctoral thesis, entitled "Investigations on the Physiology of Cells. Materials Leading to the Knowledge of the Movement of Protoplasma, Plasma Membranes, and Chloroplasts", at the University of Geneva. J. Briquet, one of the reviewers of M. S. Tswett's thesis, stressed: "I believe that this work is so serious and complicated both in its contents and in the way in which the author made it that it by far surpasses our other doctoral theses".

In the spring of 1896 Tswett left Switzerland, and after a short stay in Italy, where he visited scientific and botanical centers in Florence and Rome, he moved to Russia.

The early period of Tswett's life in Russia was not an easy one. All of his attempts to find a proper work place to continue investigations of the physiology of plants failed. This was mainly caused by the fact that his Doctor's degree received abroad was not equal to a Master's degree in Russia and gave no right to apply for a job in a higher education or research institution. That is why Tswett decided to present anew his Master's dissertation in Russia.

Tswett started working without a salary at the Laboratory of Anatomy and Physiology of Plants, directed by academician A. S. Famintsyn, at the St. Petersburg Academy of Sciences. In 1897 Tswett began working at the Biological Laboratory of St. Petersburg, founded and directed by Professor P. F. Lesgaft. Tswett became head of the botanical department and a reader in

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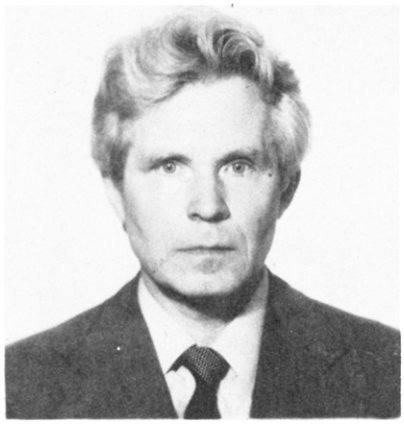

Victor G. Berezkin was born on April 18, 1931, in Moscow. He is currently Head of the Chromatography Laboratory of the Institute of Petrochemical Synthesis, USSR Academy of Sciences. He is the author of 300 papers and 12 books and has scientific interests in the areas of analytical and physical chemistry, especially, physicochemical methods of analysis, gas chromatography, and liquid chromatography.

botany at a school for women. This was the first place where he started receiving wages. At the same time he worked hard on the preparation of his Master's thesis; he carried out the experimental part of his work both at the Laboratory of Anatomy and Physiology of Plants at the St. Petersburg Academy of Sciences and at the Biological Laboratory of St. Petersburg. Gradually his mood changed for the better. In a letter to J. Briquet dated August 8, 1898, he wrote concerning an offer to come to and work in Germany: "Thank you very much for your proposal. A year ago I would have accepted it without hesitation. However, at present I have obtained a position which is as good as the one you offer me, and I believe it will be much better in the nearest future. Besides, I cannot decide on exchanging my independence for responsibilities which are, I believe, rather time- and labor-consuming. It is quite obvious to me that staying among scientists in Germany would give me some "school"—as we say here—or, in other words, the pedantry and straightforwardness in work that I lack. Well, I will have to concentrate all my will to compensate for this shortcoming".

In 1901 Tswett defended his Master's thesis at the University of Kazan. It was entitled "A Physico-Chemical Study of the Chlorophyll Grain. Experiments and Analysis". He dedicated this work to the memory of his father, who died on April 29, 1900, in Yalta. The dissertation started with the words: "This is dedicated to the memory of my father—thinker and worker—Semen Nikolaevich Tswett".

The qualifying examination was a success. Tswett's official reviewer, Professor N. V. Sorokin, stressed at the end of his review: "Everything in this work proves Tswett's hard labor. Only a man who is devoted to science, who cannot be frightened by voluminous literature, and who is well prepared for such an investigation could decide to start even a special research on chlorophyll.

These investigations have led Tswett to discover new interesting compounds contained in chloroplasts, and his critical review of previously written and modern literature proves he is a man of great learning. That is why I can say without hesitation that the work entitled "A Physico-Chemical Study of the Chlorophyll Grain. Experiments and Analysis" completely meets all the requirements claimed to obtain a Master's degree in botany".

In November of 1901 Tswett became an assistant in the Department of Anatomy and Physiology of Plants at Warsaw University, headed by Professor D. I. Ivanovsky.

However, the Master's degree did not improve Tswett's financial position. In order to support his research on adsorption chromatographic analysis he had to read lectures at secondary schools. In the summer of 1902 Tswett went on a research trip to Germany; at the same time he was elected a member of the German Botanical Society. Later, 16 articles by Tswett (most of his papers published abroad) were published in *Reports of the German Botanical Society* (*Ber. Dtsch. Bot. Ges.*).

Tswett first spoke about his chromatographic analysis at a session of the biology Division of the Warsaw Society of Naturalists held on March 8, 1903. His report, "About a New Category of Adsorption Phenomena and Their Application for Biochemical Analysis", caused a lively discussion between all those present and Tswett. The same year his report was published in the *Proceedings of the Warsaw Society of Naturalists* (*Tr. Warshaw. Obshch. Estestvoisp. Otd. Biol.* **1903**, *14*, 20–39). That is why the year 1903 is the birthyear of chromatography. Two articles describing chromatographic analysis and its role in chlorophyll chemistry were published in 1906 in *Reports of the German Botanical Society* (*Ber. Dtsch. Bot. Ges.*).

In 1907 Tswett married Helena Aleksandrovna Trusevich, who worked in the library of Warsaw University. H. A. Tswett proved to be a loving and faithful friend, and she did her best to give him comfort at home. Later, H. A. Tswett mentioned in her memoirs that on the Tswetts' engagement day she and her mother learned that M. S. Tswett had no apartment and spent nights in the botany laboratory on a table. H. A. Tswettt also participated in her hushand's scientific activities, and she helped him with his doctoral thesis.

In 1908 Tswett was accepted on the staff of the Warsaw Polytechnic Institute to study botany. Professor D. I. Ivanovsky wrote in his reviews of Tswett's works: "One cannot help marking the active role of the young scientist who managed to publish not less than 40 scientific articles during 14 years. In general, I believe that Tswett's works prove him to be an accomplished and independent scientist who is well acquainted with the methods of scientific analysis and who has already obtained a prominent position in the world of science".

In November of 1910 Tswett passed his qualifying examination for his Doctor's degree at Warsaw University. His official reviewer, Professor D. I. Ivanovsky, stressed: "Works by Tswett make a real revolution in the theory of photosynthetic pigments and provide him with an outstanding role among specialists in this field".

In the summer of 1911 Tswett visited scientific centers of Western Europe (Berlin, Paris, Amsterdam, Leyden, and Delft) to acquaint himself with methods of teaching microbiology in specialized higher institutions. This strengthened and expanded his scientific contacts with his foreign colleagues.

In 1911 the Physics and Mathematics Department of the Academy of Sciences on the initiative of academician A. S. Famintsyn awarded Tswett the N. A. Akhmatov Grand Prize of 1000 rubles for his monograph *Chromophylls in Plants and Animals*. In his review academician Famintsyn stressed that "a new adsorption method, elaborated by Professor Tswett, allowing us to explore with much more precision than by means of other known methods chromophylls in plant extracts made by different solvents, is the crown of this work".

Tswett's ability to lecture was described in the following way by Professor I. I. Bevad, the Dean of the chemistry faculty of the Warsaw Polytechnic Institute: "...M. S. Tswett is a lecturer in botany; he has been delivering lectures and directing seminars with students for a number of years, having thus proven he is a highly qualified lecturer. He has put teaching botany on a strictly scientific basis and does it at a exceedingly high level; he knows how to find the right approach to students and there has not been a single conflict between them. He is a man of high moral qualities—he is ardently devoted to duty, he is modest, and, despite his wide popularity among scientists, he is a hard-working, kind, and sympathetic person, and in general a man of very pure nature".

In 1912–1914 Tswett's health changed for the worse, which showed in his creative activities. When the First World War broke out, Tswett moved with the Warsaw Polytechnic Institute to Nizhny Novgorod, and in 1917 he was appointed professor at the University of Yuriev. In 1917 the university was moved to Voronezh. On June 26, 1919, M. S. Tswett, who suffered from heart disease, died in a hospital and was buried in Voronezh.

References

(1) Rikhter, A. A.; Krasnosel'skaya, T. A. "Role M. S. Tswetta v sozdanii chromatigraphiczeskogo adsorpzionnogo analyza i ego kratkaya biographiya" ("The Role of M. S. Tswett in the Creation of Chromatographic Adsorption Analysis and His Short Biography"). In *M. S. Tswett. Chromatographiczeskii adsorptionnyi analyz (Chromatographic Adsorption Analysis)*; Academy of Sciences of the USSR Publishers: Moscow, 1946 (in Russian).
(2) Senchenkova, E. M. *Mikhail Semenovich Tswett*; Nauka: Moscow, 1980 (in Russian).
(3) Vigdergauz, M. S. *Tswetopis* (Colorscript); Khimia: Moscow, 1980 (in Russian).
(4) Sakodynskii, K. I.; Chmutov, K. V. "M. S. Tswett and Chromatography". *Chromatographia* **1972**, *5*, 471.
(5) Sakodynsky, K. I. "The Life and Scientific Works of M. Tswett". *J. Chromatogr.* **1972**, *73*, 303.

About a New Category of Adsorption Phenomena and Their Application for Biochemical Analysis†

MIKHAIL S. TSWETT (1872–1919)

Preliminary communication, March 1903

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At present the term *adsorption* applies to a variety of phenomena that, although heterogeneous by nature all meet the same general criterion, namely condensation of gases, vapors, or liquids (or the substances dissolved in these liquids) on the surfaces of bodies that they surround.

Not only animal coal and charcoal adsorption of dyeing substances, as has been commonly believed, but also the adsorption of a variety of dissolved substances, including gases, the adsorption capacity of soils, the immense sorption capacity of gases by platinum black and palladium black, the condensation of water vapor and gases on glass and metallic surfaces (formation of so-called Wasserhaut and Lufhaut), the adsorption of water vapor by finely powdered and metallic oxides in the colloid state, the adsorption of water by the hydrogel of silicic anhydride, the sorption of salts by cellulose and animal membranes, the adsorption of dyes by vegetable and animal fibers (dyeing ability), and coloring of histological preparations are phenomena that belong either fully or partially to the category of adsorption.

Adsorption is far from being completely understood and only in rare cases do the available experimental data provide a clear choice between different existing physical and chemical theories, which try to explain some or all of the above-mentioned phenomena. In the future each of these theories will undoubtedly gain its own specific sphere of application.

Up to now adsorption of dissolved substances has been studied mainly in water medium. If we neglect certain experiments in the field of dyeing technique, where alcohol pigment solutions are used, as well as exclude the observed bleaching effect of coal on not only aqueous but also organic solutions, adsorption from organic solvents still remains terra incognita. However, we can hope that the investigation of adsorption from organic media may turn fruitful. Let us take, for instance, such dissimilar solvents as ether, chloroform, carbon disulfide, members of the hydrocarbon series, and mainly petroleum hydrocarbons (benzine, petroleum ether, ligroin). More or less absolute chemical inertness of these liquids, their weak electrodissociative action on solutes, and their poor capacity to dissolve most inorganic and many organic substances (this capacity permits study of the adsorption action of numerous adsorbents on the same substance) are all of great methodological value in an investigation.

The present work has as its starting point experiments concerning the problem of insolubility of chlorophyll from leaves in benzine or ligroin. I conducted these experiments several years ago.[1] Under the term chlorophyll, as it is generally known, we mean and should exclusively mean the group of pigments soluble in alcohol, the totality of which gives vegetation its

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specific color. This green complex has not less than five different pigments, which can be very conveniently divided into two groups: *chlorophyllins* (chlorophyllin α and chlorophyllin β, possessing red fluorescence and having a band of absorption in both halves of the spectrum, and *xanthophyllins*[2] (carotene, xanthophyll α, and xanthophyll β), yellow in color, giving no fluorescence, and having bands of absorption only in the right half of the spectrum.

All of these different pigments since they are extracted from tissues by means of alcohol (or acetone or ether) could be distinguished and to some extent separated from each other with the help of differential dissolution in biphase systems, for example ligroin + alcohol + water or carbon disulfide + alcohol + water (this method was introduced to chlorophyll studies by Stokes, Sorby, and Craus), and all of them turn out to be quite soluble in benzine and ligroin.

It was long ago shown in the literature that the above-mentioned solvents extract from fresh or dry leaves only traces of "green substances" (chlorophyllins) and that the thus-obtained solution has a more or less intense yellow color caused by carotene (according to studies by Arno).

What causes the "insolubility" of the majority of chlorophyll pigments? This phenomenon has been given different and contradictory explanations. Some authors referred to a certain imaginary inaccessibility of pigments for solvent. Others believed that the pigment remaining in the tissue (they assumed there was only one pigment) was simply not soluble in ligroin and, if once extracted from leaves by alcohol, it became soluble, which meant that it had changed. Thus Monteverde,[3] who was studying ligroin-insoluble green crystals discovered by Borodin, viewed them as being the source of the green color of chlorophyll.

The experimental studies of this question have led me to the following conclusion. The insolubility of most chlorophyll pigments from leaves in benzine and ligroin is not caused by their insolubility in these liquids but can be explained by a retarding action of molecular forces of the substrate, i.e., by the adsorption process. The dissolving energy of ligroin for these pigments is exceeded by the adsorption force of the substrate, but the latter is overcome by other solvents such as alcohol. Therefore adding even a small part ($^1/_{100}$) of absolute alcohol to ligroin causes an immediate and total extraction of all pigments. Fully lignoin-solubilized chlorophyll, having been extracted from leaves by means of evaporating under vacuum in the presence of filter paper, penetrates the latter.

Thus chlorophyll-colored filter paper reacts to solvents as if it were a green leaf. Only carotene is extracted from this paper by ligroin, and all other pigments are released after adding traces of alcohol. An experimenter can easily change chlorophyll from the "soluble" into the "insoluble" form and back without affecting the chemical nature of the pigments, as proved by spectroscopic measurements. Since the existence of any chemical affinity between the cellulose of the filter paper and chlorophyll pigments is highly improbable, we shall admit to dealing with typical adsorption phenomenon.

The latest experiments have shown (as expected) that paper immersed in a ligroin solution of chlorophyll itself turns green, as it attracts pigments in accordance with its adsorption capacity.

It also should be expected that different powdered substances will have adsorption action on pigments of chlorophyll in ligroin solutions, and there appears to be a certain hope that systematic study of this question would throw some light upon the nature of the adsorption phenomena and enable elaboration of a new technique of physical separation of substances on this basis. These considerations have motivated me to conduct an extensive series of tests, the main results of which are outlined below.

Adsorption of Chlorophyll Pigments from Ligroin Solutions

Preparation of Solutions

Pure ligroin (or benzine) is capable of extracting noticeable amounts of chlorophyllins from fresh leaves but such extracts are still relatively poor in pigments, and solutions prepared in the below-described fashion turn out to be more suitable for adsorption experiments.

(1) Fresh leaves (I used *Lamium album* and *Plantago media*) are quickly ground in a mortar with the addition of crushed glass and magnesium oxide or calcium carbonate (for removing acids). The leaves are covered with acetone and further ground, and the resulting extremely thick solution of chlorophyll is forced through a double filter paper or, better, through a dense asbestos plug under negative pressure. The obtained transparent liquid is evaporated under vacuum at 50–60 °C, and the dry residue is dissolved in pure ligroin. The resulting solution contains the original pigments, as shown by spectroscopic studies.

(2) Leaves are ground in the fashion described above but the extraction is carried out by means of ligroin mixed with $^1/_{10}$ part of absolute alcohol. After filtration the green solution is shaken with an equal amount of water into which nearly all the alcohol available in the extract is merged. The resulting ligroin layer, which is usually more or less turbid (because of suspended tiny water drops), is forced through the filter paper, and an absolutely transparent green solution is obtained.

Adsorbents

Listed below are more than 100 substances from various groups of the periodic system that have been studied as adsorbents. Thus we could hope to discover a general adsorption action independent of molecular structure and, simultaneously, to notice possible chemical effects of different substances on chlorophyll pigments.

The majority of preparations used in these studies were supplied by the German firms Calbaum, Merck, Scherring, and Grubler; others were supplied by the local factory of Rutkovski.

Simple bodies: sulfur, silicon, magnesium, zinc, iron, lead

Oxides and hydroxides: oxides of magnesium, iron, lead, silver, and antimony, manganese peroxide, aluminum hydroxide, sodium hydroxide, potassium hydroxide, barium hydroxide

Acids: boric, oxalic, tartaric, tannic, uric, picric

Salts of
- hydrochloric acid: sodium, potassium, ammonium, calcium, barium, magnesium, aluminum, iron, cobalt, copper, mercury
- perchloric acid: potassium, barium
- hydrobromic acid: potassium
- hydriodic acid: mercury
- periodic acid: potassium
- hydrosulfuric acid: potassium, mercury
- hydrosulfites: mono- and bisulfites of sodium
- sulfuric acid: potassium, ammonium, calcium, barium, magnesium, iron, manganese, copper, zinc; ammonium copper sulfate
- nitric acid: potassium, calcium, barium, copper, silver, lead, uranium
- phosphoric acid: monosodium, mono- and dipotassium, triammonium; phosphates of lime and iron
- carbonic acid: sodium, potassium, manganese, iron, copper
- silicic acid: potassium and also asbestos
- molybdic acid: ammonium
- organic acids: lead and copper acetates, ammonium and manganese oxalates, iron(III) oxide saccharate
- cyanic acid: potassium ferricyanide and potassium ferrocyanide (ferroprussiate)

Aldehydes: chloral hydrate
Amides: asparagine, urea
Polyatomic alcohols: mannitol, dulcitol
Hydrocarbons: saccharose, galactose, inuline, starch, cellulose
Benzene derivatives: resorcinol, hydroquinone, pyrogallic acid, phenolphthalein
Alkaloids: quinoline
Albuminoids: ovalbumin, peptone, hemoglobin
Different substances: carmine, fluorescein
Substances of an unknown composition: glass wool, soil, emery, blood coal, bone coal

Experimental Techniques

Adsorption tests were carried out in three different ways.

1. Adsorbent, elaborately ground to a fine powder in a carefully dried mortar, was poured into a narrow-stem funnel; a paper cap was placed on the tip of the funnel to retain the powder. The powder was densely and evenly packed with a glass stick, and then either a ligroin solution of chlorophyll was directly poured on top of the power or pure ligroin was passed beforehand through the powder to displace air. The filtration was carried out under a small negative or positive pressure.

2. The chlorophyll solution was poured into a test tube and adsorbent was added. After a thorough shaking, the contents of the test tube were subjected to centrifugal force, and adsorbent accumulated at the bottom together with adsorbed pigments. Conversely, adsorbent powder was poured into a test tube and shaken throughly with a small amount of pure ligroin; only then was chlorophyll solution added.

3. In experiments with hygroscopic substances that rapidly spread in air such as copper or calcium nitrate, copper hydrochloride, and others, the chlorophyll solution was poured into a mortar, adsorbent was added directly from a storage can, and the mixture was ground under ligroin.

Results

All of the tested substances *without any exception* were able to adsorb either part or the totality of the chlorophyll pigments. To be clear, exact, and brief, I shall first describe the results of the tests with inuline as typical in a certain sense and then outline those deviations that were observed in tests with other adsorbents.

Inuline Tests. The Calbaum preparation used in my tests is a fine powder consisting of more or less spherical particles of ~2-μm diameter. Assuming that the density of inuline is equal to 1.35 and that all particles are uniformly 2 μm in diameter, it is easy to calculate that the total surface area of the particles in 1 g of this powder is 2.22 m^2.

When the chlorophyll solution is shaken in the presence of inuline, some of the pigments are immediately adsorbed, and the powder sinking to the bottom of the test tube turns green while the liquid, which gradually clears, becomes yellow. As more adsorbent is added, the yellow intensifies; if sufficient adsorbent is added, only carotene remains dissolved in the ligroin solution. Carotene is identified by its spectrum and also by the fact that when the solution is shaken with 85% alcohol, the pigment remains practically totally in the ligroin phase.

The green sedimented powder is shaken again with pure ligroin to remove the last traces of carotene retained by capillary forces. Different solvents may be used to liberate pigments from the powder that has adsorbed them: alcohol, acetone, ether, chloroform, ligroin with admixed alcohol, and, partially, carbon disulfide. The last two solvents in pure form extract only some pigments; all pigments are dissolved entirely only when alcohol is added.

For the liberation of pigments it is most convenient and rational to use ligroin mixed with $^1/_{10}$ part of alcohol. The spectroscopic study of the green solution obtained shows that the pigments remain unchanged. When this solution is shaken with 80% alcohol, the normal Craus reaction is observed. The alcohol phase, containing predominantly xanthophylls α and β, is bright yellow, and the ligroin phase, containing mainly both chlorophyllins, is blue-green. The pigments can be transferred back to a pure ligroin solution, whence they can be adsorbed again with inuline and other adsorbents.

The adsorption phenomena are most clearly demonstrated when a substance is allowed to filter through the powder. First colorless and then yellow liquid (carotene) flows out of the lower end of the funnel, while an intense green ring is formed in the top layers of the inuline column. Soon a yellow borderline appears at the lower edge of the ring. During subsequent passing of pure ligroin through the inuline column, both the green and yellow rings are considerably widened and spread down to a certain limit. This proves that here (as in other already known cases of adsorption) the amount of adsorbed substance depends on its concentration and osmotic tension in the solution but is not proportional to the latter since it does not become zero when the osmotic tension is equal to zero.

If the filtration process is conducted through a column of powder whose length is insufficient for retaining all dyeing substances, the yellow ring in its downward

movement can reach the filter paper covering the lower end of the funnel and the outflowing ligroin is yellow again. Spectroscopic studies of the ligroin effluent show that the main pigment of the yellow ring is xanthophyll α. Differentiation also occurs in the green ring itself: there appears a blue-green lower zone and a yellow-green upper zone.

Tests with Other Adsorbents. In tests with calcium carbonate, aluminum hydroxide, sugar, and many other adsorbents, we observe the same thing as in the test with inuline. In other cases certain *deviations* from the above-described process take place.

First, many substances absorb carotene along with chlorophyllins and xanthophylls α and β. Among them we find the anhydrous sulfates of copper, iron, magnesium, and barium, the oxides of iron, silver, and lead, manganese peroxide, anhydrous calcium chloride, and bone and blood charcoal. In fact, the majority of these substances chemically destroy carotene (and other pigments). Among the others, lead oxide, silver oxide, and calcium chloride produce an exclusively physical effect.

Second, many adsorbents cause chemical alteration of the adsorbed pigments. For example, caustic alkalis act on chlorophyllins in the same way they do in solution. Another type of change that chlorophyllins undergo when they are adsorbed by many substances may be called "acidic", since the above-mentioned pigments undergo the same spectroscopic changes that are observed under the action of mineral and organic acids on alcohol extracts of chlorophyll (in these cases, the "chlorophyllan" spectrum appears). The nature of these acidic changes is quite unknown in spite of numerous experiments.

There are some indications of probable oxidation taking place here. Among adsorbents having an acidic type of action on chlorophyllins, we should name all the acids listed above excluding boric acid but adding pyrogallic acid, resorcinol, asparagine (succinaminosuccinic acid), and phenolphthalein, all salts of hydrochloric acid with the exception of salts of alkali metals, all salts of sulfuric acid with the exception of ammonium copper sulfate, all salts of nitric acid with the exception of barium nitrate, and all salts of phosphoric acid with the exception of ammonium tertiary phosphate. Manganese peroxide and potassium ferrocyanide have real oxidative activity, destroying all pigments of chlorophyll.

It is of interest that some of the salts with acidic action become chemically indifferent to chlorophyll pigments when they are deprived of water of hydration by means of intensive heating. Examples are the calcium salts of nitric and hydrochloric acid.

It should be noted here that "acidly" altered chlorophyllins (at least chlorophyllin α) are considerably less subject to adsorption as compared with the original pigments as clearly seen in filtration tests, where the observed differentiation of colored rings differs from the normal mode described above. This differentiation is described in greater detail in my more comprehensive paper.

The preparations of animal charcoal tested by me deserve special mention. Both of them are the most energetic adsorbents of the totality of the chlorophyll pigments. Particularly active is bone coal, from which adsorbed pigments could be extracted not only by ligroin admixed with alcohol but even by pure alcohol. The enormous adsorption capacity of bone coal can be explained, as already done by Quinke,[4] by the great amount of pores of this substance. The influence of these pores is equal to a crushing down of the matter that can hardly be obtained with mechanical force.

Adsorption of Chlorophyll Pigments from Benzene and Carbon Disulfide Solutions

Whereas benzine and ligroin extract insignificant amounts of chlorophyllins and xanthophylls from fresh leaves, benzene and carbon disulfide convert into soluble form the totality of these pigments. In fact, the first extracts here also do not contain pigments in the same relative proportions as in the original chlorophyll grains, since carotene and xanthophyll α are predominant. However, by means of repetitive extractions it is possible to achieve the removal of all the pigments. This fact, by the way, could be foreseen on the basis of my previous microscopic observations. The addition of a small portion of alcohol to benzine and carbon disulfide has the same effect as the addition of alcohol to ligroin. It causes an immediate transfer of the pigments, and a thick solution of chlorophyll is obtained.

As for dry leaves neither benzene nor carbon disulfide extracts from them all the dyes completely. In accordance with this, powdered substances adsorb chlorophyllins and xanthophyllins from their benzene and carbon disulfide solutions. The difference with ligroin solutions lies in the fact that to adsorb a certain quantity of pigments, a smaller amount of adsorbent is needed in the former solution than in the latter. Accordingly, the adsorbent saturated in ligroin solution would yield part of the previously adsorbed pigments to benzene or carbon disulfide.

Adsorption of Different Substances from Organic Solutions

There can be no doubt that, in addition to chlorophyll pigments, many other substances soluble in ligroin, benzene, and carbon disulfide would be submitted to the adsorption process from these media. In this connection it would be of interest to choose a few such substances that would be chemically definite and available in pure form and whose solubility laws in different liquids would be known and easily subjected to analysis.

Having a series of such objects, we could hope to start a successful study on the adsorptive function, which depends on the properties of the solvent and the adsorbent as well as the properties of the dissolved substance.

Cyanin (quinoline blue, $C_{30}H_{39}N_2I$) is apparently quite suitable for adsorption tests. Hardly soluble in ether and ligroin, cyanin is easily dissolved in benzene and is adsorbed from it by calcium carbonate, sodium carbonate, lead oxide, saccharose, etc. Addition of a few drops of alcohol releases the pigment from the benzene solution.

Alkannin ($C_{15}H_{14}O_4$) in ligroin solution is not adsorbed by calcium carbonate but is retained by lead oxide in the same way as carotene.

A red pigment available for sale as *sudan III* reacts with respect to adsorbents as an alkannin.

I have also tested *lecithin*. The test was carried out in the following way. A fresh egg yolk was shaken with ligroin and $^1/_{10}$ part of alcohol. A granular residue and transparent yellow liquid were obtained. The latter was decanted and evaporated on a water bath under vacuum, and the resulting oily residue was dissolved in pure ligroin.

A few cubic centimeters of the solution obtained was passed through an inuline column. First, the liquid flowing out of the column was colorless, and then it turned yellow and contained an oily substance. A flow of pure ligroin through the column was maintained for a long time until the outflowing liquid ceased to be yellow and would not give a transparent oily spot when evaporated on silky paper. At this point ligroin admixed with alcohol was passed through the column. A pale yellow solution started flowing out, and the dry residue of this solution appeared to be a waxy substance that possessed birefringence properties, swelled in water, yielded micelles, and liquefied in a concentrated aqueous solution of resorcinol—in other words, demonstrating properties typical of lecithin.

This test shows that lecithin is adsorbed from ligroin solution and that this property could be used for its separation from the usually accompanying fatty substances.

Adsorption Analysis

On the basis of the foregoing, a new method of physical separation of different substances in organic media can be proposed. The principle of this method relies on the property of dissolved substances to make physical adsorption compounds with various organic and mineral solid materials. The amount of the dissolved substance found in the adsorption compound with a certain amount of adsorbent depends on the degree of grinding the latter, as well as on its nature, on the nature of the dissolved substance, and on the nature of the solvent. All of these differences could be used for the separation of the substance by means of their fractional adsorption precipitation.

As an example let us apply the new method to chlorophyll analysis.

Finely powdered calcium carbonate, which is an indifferent adsorbent, is added in successive portions to the ligroin solution of chlorophyll. We add the powder until the solution, after shaking and centrifugation, is pure yellow without any traces of red fluorescence. I conducted this fluorescence analysis in my fluoroscope. The green precipitate of calcium carbonate is shaken once more with pure ligroin until the latter comes to the surface colorless. The washing portions are then combined with the yellow liquid described above.

This liquid undergoes again the process of adsorption precipitation with the excess of calcium carbonate powder. After centrifugation the yellow liquid is decanted. The yellow precipitate is washed again with pure ligroin, and the liquid obtained from the column is combined with the first yellow solution. The combined solution has the spectrum of pure carotene: I, 475–492 nm; II, 443–460 nm; III, 420–430 nm. The yellow precipitate is shaken with ligroin and $^1/_{10}$ part of alcohol and becomes colorless. The yellow solution gives the following spectroscopic data: I, 470–480 nm; II, 440–452 nm; III, very weak band at ~425 nm. This is the spectrum of *xanthophyll* α. If the solution is shaken with 80% alcohol (after Craus), the ligroin phase becomes nearly colorless, while the alcohol phase becomes yellow and gives the same spectrum.

As for the primary green precipitate the pigments are extracted from it by means of alcoholic ligroin, and this solution undergoes differential dissolution in the manner described by Craus. The ligroin phase contains most of the chlorophyllins α and β without an admixture of carotene. In the alcoholic phase there are traces of chlorophyllins α and β (chlorophyllin β predominates), xanthophyll β, and a certain amount of xanthophyll α. The separation of xanthophyll β could be carried out in the manner suggested by Sorby, in which the alcoholic phase is shaken repeatedly in the new portions of ligroin and every time a small amount of water is added. With each step the alcoholic phase is enriched in xanthophyll β, and its spectrum approaches that of xanthophyll β: I, 462–473 nm; II, 435–447 nm.

Reviewing the just-described analysis of chlorophyll, we can see that the adsorption method applied to it allows (1) the quantitative separation of carotene and (2) separation of xanthophyll α in a pure state, which makes it easier to identify chlorophyllins. Without any doubt further investigation on the mechanism of adsorption would lead to the perfection of its analytical application. The empirical determination of the adsorption properties of different substances found in living organisms or soluble in organic liquids would lead to the elaboration of certain adsorption–analytical approaches for various practical problems.

References

(1) Tswett, M. S. *Tr. Kazansk. Obshch. Estestvoisp.* **1901** *XXV* (in Russian). *Proceedings of the Kazan Society of the Naturalists* **1901**, *XXV*.

(2) (a) Sorby, H. *Proceedings of the Royal Society of London* **1873**, *23*. (b) In my cited work I assumed the existence of only two xanthophyllins: *carotene* and *chrizophyll*. But here also, as in the case of the double nature of chlorophyllins, my further research has fully confirmed the observation made by Sorby ("On Vegetable Chromatology". *Proc. R. Soc. London* **1873**, 23). Chrizophyll (Craus and Monteverde xanthophyll) appeared to be a mixture of two pigments differing among other things, in the relative positions of their absorption bands. I choose calling the pigments xanthophylls α and β, with α referring to the pigment having its absorption band closer to the red part of the spectrum.

A few words about Sorby's work are in order. The author of this remarkable work, who has remained unrecognized and forgotten for nearly three decades, should be given credit at least now. In view of the appearance in the newest literature of the completely erroneous evaluations of the works by Markhlevsky and Shenke Jr., I should like to remind readers that in the 1870s Sorby proved quite correctly both the duality of chlorophyllins and the triplicity of xanthophyllins. The most recent authors can be merited only with more or less successful *repetitions* of Sorby's experiments.

(3) Monteverde, *Acta Forti Petropol.* **1893**, *13*, 123.

(4) Quinke, G. *Pogg. Ann.* **1859**, *18*, 336.