ELSEVIER

Biophysical Chemistry 108 (2004) 9–16

Biophysical Chemistry

www.elsevier.com/locate/bpc

Those wonderful early years with the Model E ultracentrifuge and David Yphantis

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1. Before the Model E

As a newly minted faculty member in 1948 with the auspicious title of 'Instructor', I was the proud recipient of one of the early Model E ultracentrifuges produced by the Specialized Instrument Corp. (Spinco). This was a superb gift from Wendell Stanley who had just moved to the University of California in Berkeley as Chair of the Department of Biochemistry and Director of the Virus Laboratory. Fortunately for me, in his leaving the Department of Animal and Plant Pathology at the Rockefeller Institute for Medical Research in Princeton, New Jersey to assume his new position, Stanley offered me the opportunity to accompany him. I had been a technician in his lab working primarily with Max Lauffer on various physical–chemical problems aimed at determining the homogeneity, sub-unit structure, size and shape of viruses. With their generous assistance, I was also a part-time graduate student in the Department of Chemistry at Princeton University working with Henry Eyring. Following a stint in the Navy during World War II, I returned to Princeton where I obtained my Doctorate in 1948 as Walter Kauzmann's first graduate student. To this day, he denies that I was his student—clearly he is making a telling statement.

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At the Rockefeller Institute, the (trouble-plagued) air-driven ultracentrifuge was the major tool for studies of tobacco mosaic virus (TMV) and a host of other viruses targeted for research during World War II. For those who used to complain (and still do) about inadequacies in the Model E or its successors, the XL-A and XL-I, I can only wish that they had experience with one of the roughly half-dozen air-driven instruments in the world. Obtaining a vacuum seal between two large flat surfaces required a delicate touch with the proper lubricant, and it constituted a major challenge. Much too often my technique proved unsatisfactory. There was either too much grease or too little. In each case, the flat gasket was 'sucked' into the chamber when the vacuum pumps began operating. The later development of the O-ring and a groove to contain it in the Model E solved that problem in a totally reliable fashion. It is difficult now to imagine a world without O-rings. Those few air-driven ultracentrifuges were the only alternatives to the oil–turbine ultracentrifuge designed originally by Svedberg et al. in Uppsala [1], and there were even fewer of these available anywhere. Hence, the application of the ultracentrifuge for the study of biological macromolecules and synthetic high polymers was restricted to only a few laboratories throughout the world. The subsequent introduction of the commercially available Model E constituted a revolution in attracting many imaginative and productive biochemists and physical chemists to using the ultracentrifuge for research

0301-4622/04/$ - see front matter
doi:10.1016/j.bpc.2003.10.005

in the burgeoning field of macromolecular biophysics.

2. The Model E appears

So there I was with my reliable Model E and a sensational graduate student, Bill Harrington, using the ultracentrifuge day and night in studies of the sub-unit structure of TMV. By and large with few exceptions, my colleagues in Berkeley had little interest in macromolecules or the tools used to study them. However, there were compensations in that I could collaborate with some faculty members on biological problems. Being curious about the macromolecular organization of microbial cells, I was able to convince Arthur Pardee and Roger Stanier that it would be worthwhile to look at cellular extracts in the ultracentrifuge [2]. This effort led to the discovery of the 30 S and 50 S particles, now known as ribosomes. Later with Chao, we discovered the effect of magnesium ions in promoting the formation of 70 S particles in yeast [3]. Also, experiments with absolutely opaque extracts from photosynthetic organisms showed that all of the pigments of the photosynthetic machinery were contained in large particles with a sedimentation coefficient of approximately 200 S, which we named chromatophores as the analogue of chloroplasts [4].

Despite the absence of others interested in the theory or application of the ultracentrifuge, Bill and I had a very productive few years focused largely on applying the instrument to study the subunit structure of TMV. But then, in some control experiments, we came across a bizarre finding leading to a major change in our research. In the analysis of known mixtures, the concentrations determined from the areas of the peaks corresponding to the different components were not what they should have been [5]. This anomaly for mixtures had already been considered and interpreted by Johnston and Ogston [6] in an important paper that was hard to read because of a mix-up of figure legends and figures. Moreover, their interpretation was considered by some (of the Svedberg school) to be controversial and did not receive the widespread acceptance it merited. For a quantitative treatment of the problem, it was necessary to determine the sedimentation coefficient of the slower component in the presence of the faster one. Bill and I approached that dilemma by layering a solution of the faster component over another (more dense) solution of the mixture, thereby establishing a detectable boundary due to the slower component. Although this layering in a cell on the laboratory bench was not difficult, the rotor then had to be reoriented through 90°, and the hard won boundary was virtually destroyed. Accordingly, I approached Ed Pickels at Spinco pointing out the need for a special ultracentrifuge cell to facilitate the layering of one solution over another in the spinning rotor. This visit led not only to a working synthetic boundary cell but also to a long lasting and fruitful collaboration.

Ed Pickels, as a physics graduate student in Jesse Beams's laboratory at the University of Virginia, had played a major role in the design and construction of the air-driven ultracentrifuge [7]. In the midst of a very successful career in the laboratories of the Rockefeller Institute in New York, he moved to California to become a co-founder and chief scientist at Spinco. Within a few years the Model E, based on his ingenuity, was developed and manufactured. I take particular pleasure in that outcome, since in a small way I was the mid-wife of that company. While in the Navy, I had met Morris Hanafin during my search for someone to build an ultracentrifuge. He was already involved in scientific instrumentation and informed me of his interest in entering the centrifuge business. I recommended that he contact Ed Pickels. That 'introduction' did the trick; and the two of them, with several others, started a company which has had a profound impact on biomedical research ever since.

Shortly after visiting Ed at Spinco, Bill and I had some preliminary versions of layering cells until we finally settled on one having a cup and a small rubber plug which acted as a valve [8]. Totally independently Kegeles [9], motivated by a paper by Lauffer and Taylor [10] aimed at determining the hydrodynamic volume of macromolecules, described a different boundary forming technique. Kegeles (to his friends he was Keg) modified an ultracentrifuge centerpiece so as to permit the layering of two solutions under the influence of the

centrifugal field. Having a synthetic boundary cell available in our laboratory provided an opportunity for a variety of exciting experiments. As a result, my research and that of my students for the following few years was focused on ultracentrifugation rather than the application of the instrument for the study of biological macromolecules. During that period, John Gofman and his students were doing very important research on lipoproteins following their clever analysis of the bizarre ultracentrifuge patterns observed with serum in solutions of densities causing both sedimentation and flotation of different species [11]. Interactions with that group led to Verne Schumaker becoming a postdoctoral fellow in my lab where he did some superb research on DNA, and to extensive collaboration with Rodes Trautman.

3. Sedimentation coefficients, the temperature problem, and Dave's contribution

Within a few years, Model E ultracentrifuges were being used in many laboratories thanks to funding agencies such as the National Institutes of Health and the National Science Foundation. Determining sedimentation coefficients of proteins was the initial goal in many laboratories, and efforts were focused on increasing the precision of measurements and comparing results of different investigators. Much to the dismay of some workers, values from studies of some proteins in the Model E differed by as much as 10% from earlier results obtained with the oil–turbine instrument in Uppsala. To some, the 'gold standard' was the Svedberg instrument, and questions were raised as to the cause of the discrepancy. Since it was known that the viscosity of water varies approximately 2% per degree, it seemed logical that an error in the rotor temperature in either of the instruments was the culprit. At that time, the rotor temperature in the Model E was measured before the experiment and then again at the end by means of an external contact thermocouple in conjunction with a galvanometer. Temperature increases in typical experiments were approximately 1 °C/h. The average temperature was used for corrections of the measured sedimentation coefficients to standard conditions represented by the density and viscosity of water at 20 °C. In contrast, the measurement of temperature in the oil–turbine ultracentrifuge used a much more elaborate system because of the heat generated by the bearings and friction in the relatively low-pressure hydrogen atmosphere. The different results from the two types of instruments led several workers with access to both types of instruments to focus on the problem. This activity revealed errors in the calibration of some thermocouples in the oil–turbine instruments thereby accounting for the discrepancy.

During this period Dave Yphantis, as a graduate student in David Waugh's laboratory at MIT, became interested in measuring the temperature of the spinning rotor rather than being satisfied with the 'before and after' measurements. Accordingly, he designed a 'radiation' detector providing a continuous indication of the temperature of the rotor along with a circuit and heating coil for maintaining a constant value throughout the experiment [12]. With this device, he was able to demonstrate the adiabatic cooling of the rotor due to stretching during its acceleration to 60 000 rpm. Thus, the early values from the Spinco ultracentrifuges needed to be revised slightly. Pickels, aware of the major discrepancy with the results from the oil–turbine instruments, designed an alternative sensing and control device for the Model E which provided direct measurement and control of the rotor temperature.

About that time, my occasional trips to the east coast and visits with Dave Waugh brought me in direct contact with Dave Yphantis and Pete von Hippel, who was also a graduate student in that lab. The resulting friendships have continued for more than 50 years. Following their direct measurement of the temperature of the rotor and the demonstration of adiabatic cooling, the two Daves turned to other important centrifuge problems, and their interests and mine began to intersect and overlap frequently. There was always something exciting to talk about when we met.

4. Sedimentation of dilute solutions and separation cells

In the early 1950s, we became interested in DNA and recognized immediately that ultracentri-

fuge studies using schlieren optics were totally inappropriate because of the non-ideality of solutions at the nucleic acid concentrations required for detection. Hence, I approached Ed Pickels again urging him to resuscitate the long discarded absorption optical system (with ultraviolet light) that had been devised in Svedberg's laboratory when the ultracentrifuge was being developed. In almost no time, Spinco provided a working optical system, and Verne Schumaker and I were doing experiments at microgram concentrations of DNA rather than at milligram levels [13]. As usual, there were some skeptics who maintained that convective disturbances would distort or destroy any moving boundary thereby invalidating experimental observations. But they had not considered the stabilizing effect of the density gradient resulting from redistribution of buffer constituents in high centrifugal fields. Hence, very soon the ultracentrifuge was being used routinely with absorption optics for studies of extremely dilute solutions of nucleic acids. This revival of absorption optics was also invaluable for studies of the association–dissociation behavior of hemoglobin at great dilution, since it was possible with the appropriate interference filters to exploit the intense absorption due to the Soret band. Even colorless proteins at microgram levels are readily studied by using light of wavelengths of approximately 2300 Å. For entirely different purposes, Dave Yphantis and Dave Waugh were also confronting experiments with very dilute solutions and they demonstrated that convection was not a problem.

In the course of their theoretical analysis of transient solute distributions, Yphantis and Waugh became interested in using biological activity measurements for characterizing various substances by ultracentrifugation [14]. This research led to the design of a superb separation cell with a moveable platform, which enabled them to fractionate the contents of the cell and use biological activity measurements for determining sedimentation coefficients. As one who had used the fixed partition cell designed by Tiselius et al. [15] many years earlier, I was most impressed by the moveable partition cell and was extremely grateful when Dave Yphantis kindly furnished one. I shared his view that the ultracentrifuge could and should be used in conjunction with biological activity measurements and that the reliance exclusively on optical methods was not advisable. In the early 1950s, we used the fixed partition separation cell along with P^{32} measurements to study the state of the parental phosphorus during the reproduction of T2 bacteriophage. Within the virus, the P^{32} exhibited a sedimentation coefficient of approximately 800 S. After the DNA entered the bacteria, the sedimentation coefficient decreased to approximately 20 S. Then due to the breakdown of some of the DNA to oligonucleotides, the sedimentation coefficient decreased to approximately 1 S. Later in the latent period, as the parental phosphorus was converted into progeny DNA and ultimately virus was assembled, the sedimentation coefficient increased accordingly. Thus, the life cycle could be followed using the ultracentrifuge equipped with a separation cell [16]. Hence, the appearance of the paper by Yphantis and Waugh some years later had a great impact on me. I also found their cell invaluable in preliminary experiments with Arthur Kornberg in showing that the P^{32} of deoxynucleoside triphosphates had been converted by DNA polymerase into macromolecules with the sedimentation coefficient of calf thymus DNA. This was possible in an early phase of Kornberg's research on the enzymatic synthesis of DNA when only a trace amount of substrate had been converted into product. As Yphantis and Waugh pointed out, sedimentation velocity experiments can be most productive even with impure solutions as long as there is a specific biological assay for the substance under investigation.

5. Dave's various moves and settling in Connecticut

When Dave left MIT, my contacts with him decreased temporarily only to be renewed when he went to the Rockefeller Institute (then about to be Rockefeller University) where I knew many of his distinguished colleagues. As Ed Pickels had done many years earlier, Dave exploited the superb instrument shop at Rockefeller, and he developed the multi-channel, short column centerpiece which, in one form or another, has been used for years by workers doing sedimentation equilibrium experi-

ments. Before moving to Storrs, Dave joined the faculty at the State University of New York in Buffalo where he remained for only a few years. I can still recall vividly my intemperate outburst to my friend who had recruited Dave to Buffalo when he informed me that Dave was to become Chair of the Department of Biology. From my point of view, it was virtually a crime to take Dave away from the lab bench and the ultracentrifuge and to inflict bureaucratic paper work on him. I doubt whether Dave heard my vituperative remarks, but I was ecstatic when I learned that he abandoned that position promptly and moved to the University of Connecticut. Although I did not know much about that university, I was an old friend and admirer of Keg with whom I had many pleasant and fruitful interactions while he was still at NIH before his move to Clark University and then to the University of Connecticut. Hence, with Keg already there, Storrs seemed a great spot for Dave, and he clearly thrived doing magnificent research and training a group of outstanding students.

6. Sedimentation equilibrium and interference optics

For roughly the first decade of its existence, the Model E was used almost exclusively for sedimentation velocity experiments. A small number of us used the Archibald method for several years following his very clever theoretical paper. Because sedimentation equilibrium experiments, as described originally by Svedberg and his colleagues, required so much time for the attainment of equilibrium, this method was hardly being used in any laboratory. But the neglect of this powerful technique changed abruptly as a result of the paper by van Holde and Buzz Baldwin [17] emphasizing that the time to reach sedimentation equilibrium was reduced drastically by using short liquid columns. With his multi-channel centerpieces at hand and the insight to recognize the power of the method, Dave became a leading advocate of the sedimentation equilibrium method for the study of proteins [18].

During this period, we were interested in measuring the effect of ligands on the sedimentation coefficient of proteins and recognized the need for a technique permitting the measurement of the small difference directly. It was clear that the experimental errors in two separate determinations of the sedimentation coefficients were greater than the small change we were attempting to measure. Hence, Glen Richards and I focused on the adaptation of the Rayleigh interferometer to the ultracentrifuge so that the concentration vs. distance profile of the protein in the absence of ligand would be subtracted optically from that of the liganded protein [19]. Incorporating Rayleigh optics onto the Model E proved relatively straightforward. The slight modification of the ultracentrifuge permitted the use of both interference and schlieren optics in the same experiment. After experimenting with various Rayleigh masks and alignment procedures (this activity extended over many years), we were able to measure 1% changes in sedimentation coefficient directly with high precision. Moreover, it was clear that the Rayleigh interferometer provided much more accuracy for the measurement of concentration distributions than did the more commonly used schlieren optical system. Hence, we exploited the optical system for a variety of ultracentrifuge applications including, of course, sedimentation equilibrium [20,21].

In a short time the workers, who previously had been using only the schlieren optical system, adopted Rayleigh optics. Our focus was on relatively low-speed equilibrium experiments leading to only approximately a three- to fivefold increase in concentration from the meniscus to the bottom of the liquid column. Such experiments, which are ideal when the concentration distribution is analyzed by schlieren or absorption optics, required a method for ‘labeling’ the fringes when the Rayleigh optical system was employed. This was accomplished by calculations based on the conservation of mass and the known original protein concentration (in fringes) determined with the synthetic boundary cell. Alternatively, we located the hinge point by using the ‘white-light’ fringe. These low-speed sedimentation equilibrium experiments produced results of great accuracy, but the procedures proved very tedious.

Labeling of the fringes as a measure of protein concentration was completely obviated by Dave Yphantis in his magnificent paper describing high-

speed sedimentation equilibrium experiments [22]. At the centrifugal fields used in these studies, the concentration at the meniscus was virtually zero, and counting fringes yielded directly the absolute concentration. Moreover, the measured molecular weights corresponded to very low protein concentrations. Dave's paper, which was very detailed, complicated and long (not easy to read while lying in bed), was subjected to a scathing review to the effect that 'it can't be done'. Hence, I was called by the Editor of the journal to 'arbitrate'. For me, it was easy to reach the definitive conclusion that the paper be published, despite the reviewer's remarks. My concern was that the Editor, in compromising with the critical reviewer, would attempt to convince Dave to shorten the paper drastically. Fortunately, my stressing the need to furnish all the details prevailed. As a consequence, some of the harm that would have resulted from the inappropriate application of the method was avoided, and Dave's classical paper on high-speed sedimentation equilibrium was published in what some would term 'excruciating detail'. From then on, investigators in laboratories throughout the world have been performing highly productive sedimentation equilibrium experiments by the method that Dave devised almost 40 years ago!

7. I have another love affair but Dave remains faithful

During the period that Glen Richards and I were working on adapting the Rayleigh interferometer to the Model E, I was also getting exasperated with the archaic absorption optical system that had enabled us to make the transition of applying the ultracentrifuge to the study of nucleic acids. Absorption optics was particularly appealing because its sensitivity and selectivity permitted one to attack a host of biological problems. Over time, we accumulated a family of interference filters for ultracentrifuge experiments with light of various wave lengths. Despite the versatility of the absorption optical system, I often described it with invectives including 'inflexible, inaccurate, inconvenient, laborious, time-consuming and even impossible'. Unfortunately, I was unable to persuade Ed Pickels and his colleagues to work on a photoelectric scanning absorption optical system, because they were so heavily engaged at that time in building an amino acid analyzer. Hence, we undertook that task in our laboratory.

After several years of creative and often frustrating work by Sue Hanlon, Frances Putney, Izchak Steinberg, Bob Johnson, Ken Lamers and George Lauterbach, we had a fairly reliable automatic split-beam scanning absorption optical system in place of the obsolete photographic method [23]. That was followed rapidly by the incorporation of a monochromator [24] through collaboration with Lee Gropper of the Spinco Division of Beckman Instruments, Inc. The scanner in conjunction with the monochromator proved invaluable for sedimentation velocity and sedimentation equilibrium experiments on proteins at the microgram level [25]. Moreover, the ability to distinguish different chemical species provided a powerful tool for the analysis of interacting systems. Izchak Steinberg and I used absorption optics to measure the distribution of methyl orange and Rayleigh optics for the distribution of bovine plasma albumin in a sedimentation equilibrium experiment aimed at studying their interaction [26]. Cross plotting the concentrations of the two species at conjugate radial positions yielded the data for the determination of the free methyl orange and the amount bound as a function of protein concentration. In effect, the ultracentrifuge was being used to perform equilibrium dialysis experiments; albeit at a somewhat higher cost.

One of the most satisfying applications of the scanner was the result of a discussion at a party where I learned from David Samuel of the Weizmann Institute of Science how they made pure H_2O^{18} in Israel. Upon discovering that the process led first to D_2O^{18} which was then converted into H_2O^{18}, I asked David if he could intercede and supply us with a few milliliters of virtually pure D_2O^{18}. With his kind, precious gift, Stuart Edelstein and I were able to determine both molecular weights and partial specific volumes of various substances with only microgram amounts [27].

With the various modifications of the Model E, we could do all sorts of biochemistry at $300\,000 \times g$s, and friends like Pete von Hippel came to Berkeley to collaborate and exploit our

instrument. In only a few days after being approached by John Gerhart, he and I discovered that aspartate transcarbamylase (ATCase) was composed of distinct catalytic and regulatory subunits with the latter containing the bulk of the reactive sulfhydryl groups [28]. It was fun to be able to determine the number and location of the sulfhydryl groups with the ultracentrifuge [29]. Moreover, with the difference sedimentation technique we demonstrated that the sedimentation coefficient of the enzyme actually decreased upon the binding of substrate analogues despite the increased molecular weight and density [30,31]. Thus, I became infatuated with ATCase and allostery, and that love affair continues to this day.

Meanwhile, Dave and a host of gifted students continued their pioneering research on the ultracentrifuge. Through their efforts, laser light sources appeared, computers became indispensable, new data collection and calculation procedures were developed, and the National Analytical Ultracentrifuge Facility was established at the University of Connecticut. Fortunately, I still received invitations to visit and participate in some of their symposia. So our meetings with one another, though less frequent, continued.

Then along came the XL-A. This development required sessions at Spinco, providing many opportunities for Dave and me to meet with other users, along with engineers and officials at Beckman. In that way, we were able to contribute to and influence the design, requirements and specifications of this radically new version of the ultracentrifuge. The XL-I was the next logical development. Although the XL-I is being used in Berkeley for research on ATCase mutants and colleagues are using it extensively, we no longer do research on the instrument itself. Fortunately, research on the ultracentrifuge, new ways of treating and analyzing the data, and its applications to a host of biological problems continues unabated by Dave, his former students, some old-timers and newcomers to the field. As a result, the ultracentrifuge, now more than 75 years old, is still a supreme tool for studying macromolecules and their interactions. I fully expect that Dave and his former students will continue to produce so many innovations and improvements that, at its 100th birthday, the ultracentrifuge will still be an indispensable tool for biological research.

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