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Liquid Drop Stability for Protein Crystal Growth in Microgravity

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Scientific and Technical Information Branch

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TECHNICAL PAPER

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INTRODUCTION

The growth of large, high quality protein crystals is important to biomedical research. Large crystals can be analyzed using x-ray crystallography, allowing construction of a three-dimensional model which can be used to determine the protein structure and active sites. It is then often possible to modify the protein or to develop binding compounds, leading to the development of new drugs. The large growth volumes which can be constructed in a weightless environment permit the formation of fewer and possibly larger high quality crystals than can be grown terrestrially.

Protein single crystals are needed which are of sufficient size and quality to allow the protein's three-dimensional structure to be obtained by x-ray diffraction. The key to the activity of a protein is the active site(s) formed by the conformational folding of the macromolecule. The structure of biological molecules provides insight into the function of the molecules and may also be used to design and produce new enzymes which may be structurally similar to certain proteins and can mimic the native proteins' activities. In addition, with a structural understanding of the active site of a particular enzyme, new drugs may be synthesized which can enhance or inhibit the activity of that enzyme. In practically all cases, the inability to obtain crystals of the appropriate size and quality is the limiting factor in determining the structure. Often protein crystals are of poor quality or are too small for analysis. They also tend to be quite fragile.

Experimental results from crystals grown in microgravity indicate that the more quiescent growth conditions found in space may be beneficial in growing crystals of materials which are difficult to crystallize on Earth [1-7]. Generally, in terrestrial crystallizations the crystal falls to the bottom of the crystallization container as it grows, where it does not experience an isotropic environment. There is also the problem of convective solutal flow around the growing crystal which may be quite turbulent on a micro scale [1-4,8]. In a weightless environment, neither convective flow nor crystal sedimentation occurs. In addition, in microgravity there is the possibility of doing containerless or almost containerless growth which, by providing less surface for crystal nucleation to occur, might result in fewer, but larger, crystals.

Currently, protein crystals are being grown in space by deploying a solution of protein and small amounts of precipitant from a syringe, forming a drop as large as 80 μl on the syringe tip. The drop is then exposed to a solution containing a high concentration of precipitant. Equilibration of the two solutions by vapor transport results in an increase in protein/precipitant concentration in the drop leading to protein crystal nucleation and growth. Ideally, the drop exposes as much of its surface to the precipitant solution as possible. The drop must also adhere to the syringe tip so that crystallization occurs by vapor diffusion and the drop containing crystals can be withdrawn into the syringe at the end of the experiment. The exact design of the syringe tip plays an important part in this process, and the results of research aimed at designing an optimum tip are presented in this paper. A variety of tests have been performed on drop deployment in a weightless environment using the NASA KC-135 low-gravity simulation aircraft, and the drop dynamics and stability have been analyzed.

EXPERIMENTAL PROCEDURES

The experimental environment of the NASA KC-135 has been described elsewhere [9]. In brief, the aircraft flies a series of parabolic (Keplerian) trajectories, each of which yields ~25 sec of simulated low gravity (1×10^{-2} g). A flight profile is shown in Figure 1. A typical mission of 2 hr will consist of flying 30 to 40 parabolas, which can be flown consecutively or separated by sufficient time to alter the experiment. Test systems can be either bolted to the aircraft floor or, if an effective gravitation force lower than 10^{-2} g is required, free floated inside the cargo area. In order to facilitate drop deployment, the current series of test packages were bolted down.

The basic experimental apparatus consisted of a base plate on which were mounted a 16-mm motion picture camera and two syringes with tips facing each other. This arrangement allowed drops to be deployed sequentially or simultaneously, and a variety of tips could then be compared directly under identical conditions. The tip position could be adjusted, allowing simultaneously deployed drops to be mixed if desired, demonstrating drop stability during both deployment and mixing.

A variety of tips were studied, with the following three types proving to be most interesting: a straight polypropylene tube with an o.d. of 4.0 mm and an i.d. of 1.9 mm; an 8.9-mm-diameter porous glass fritted disk glued to the front of a similar straight tube; and a smaller straight tube with the end flared to an o.d. of 1.4 mm and an i.d. of 1.1 mm. Both clear and dyed fluids were used so that drop mixing could be observed. Drop deployment and mixing using a variety of tips and fluids were observed and photographed over approximately 120 low-gravity maneuvers. Of those recorded, 11 proved suitable for further analysis.

EXPERIMENTAL RESULTS AND ANALYSIS

The main purpose of this study was to determine the syringe tip design which would deploy and hold the drops as stable as possible in a microgravity environment under realistic experimental conditions during protein crystal growth. To do this, data were analyzed from films of the tips described above, deploying drops under low-gravity conditions on the KC-135 aircraft. The approach taken was to model the drop with a dynamic system and use the model to calculate the restoring force or spring constant for the different syringe types. Drops were selected that had detectable oscillations and stayed on the tip long enough for measurements to be made.

Use was made of a film projector which could advance the film frame by frame and which had a magnifying zoom lens. The drop image was projected onto graph paper and traced and its dimensions calculated using the known syringe tip sizes as reference. The fluid densities were known, allowing the drop mass to be calculated by assuming the drop to be spherical. The contact angle between the drop and the syringe tip was also obtained from these drawings.

The film was then advanced frame by frame and the number of oscillations counted. Using a known film rate of 24 frames/sec, the frequency of oscillation (w) was determined. Eleven drops were so studied, six on the straight tip, three on the disk tip, and two on the flared tip.

The drops were then analyzed using the three model dynamic systems shown in Figure 2. The first system is a simple pendulum with a linear rotational leaf spring. As can be seen from the drawing, the moment produced by such a system is directly proportional to the angle through which it rotates. The dimensional parameter of interest, r_1, is the distance from the syringe tip to the center of mass of the drop.

The second system consisted of a pendulum with a linear coil spring attached to the mass. The differential equation derived from this system was linearized for small angles, leaving an equation of motion for a mass and spring without the pendulum, whose radius played no part in the equation for the spring constant.

The third system was a pendulum modified as shown in the figure, allowing two radial parameters in the spring constant equation, r_1 and r_2, where r_1 is defined above and r_2 is half the syringe tip diameter. This third system was designed to consider the various tip diameters.

The spring constants were then calculated by applying these model systems to the various observed drop and syringe tip combinations. Plots were made of the normalized spring constant versus mass, normalized spring constant versus contact angle, and frequency and mass versus contact angle. Since there were only two data points for the flared tip, it was neglected. The results are shown in Figures 3 through 7.

The stability and mixing behavior of the drops in microgravity are illustrated in Figure 8; this sequence shows the combination of water and dye drops during two KC-135 parabolas. The drops are quite stable, and the mixing of the solutions in each drop is shown in its initial stages. The rapid incorporation of the dye solution into the water drop is probably due to the difference in interfacial energy between the drops. Other mechanisms for this effect are also being evaluated. Using the plots shown in Figures 3 through 7, it is possible to draw some conclusions on optimum syringe tip design.

CONCLUSIONS

Before any conclusions on tip design can be reached, it is necessary to decide which dynamic model best represents reality. The first model is both simple and gives a good fit to experiment. Furthermore, it does not need to be linearized for small angle approximations. The second model, a simple mass and spring, does not appear to represent the actual drop very well. The linearization procedure used does not model the pendulum radius, which in effect is canceled out of the spring constant formula.

The third model would be useful if tip size rather than shape was the main parameter of interest, but, with the restrictions on this experiment during shuttle operations, tip shape will be the main variable dimension. This model is so dependent on size that it is not particularly useful. Our conclusions will therefore be based on the first model dynamic system, whose results are shown in Figures 3, 6, and 7.

The next item of interest is the main criteria which will be important in specifying a stable system. It was apparent from careful observation of the aircraft flight films that the higher the drop dynamic frequency, the more stable the drop. This is consistent with the theoretical model, since, for a constant mass, the frequency increases as the spring constant increases. A higher spring constant means a higher restoring force, which would increase drop stability.

It is therefore easy to see from the plots in the figures that the disk tip gives the best stability. Figure 3 shows that for a given mass, the disk tip has almost twice the spring constant (restoring force) of the straight tip. Figure 6 shows that for a constant angle, there is a marked increase in spring constant for the disk over the straight tip. Figure 7 shows that this result should be expected, since the contact angle can be seen to be a function of the mass. Physically, this result is due to the larger surface area of the disk tip.

Figure 7 also shows that for constant contact angle, the drop dynamic frequency for the disk is much higher than for the straight tip, again verifying the disk as the better tip. This result arises because, for a given mass, the disk contact angle will be lower than that for the straight tip since the disk has more area touching the drop. It may thus be concluded that for protein crystal growth in space, drop deployment systems should use a disk tip for maximum drop stability during spacecraft operations.

However, there are other considerations for flight design which make a modification of this tip necessary. While the disk design has the most stability of the tips tested, its large surface area and tip surface roughness promoted undesirable excess nucleation. Furthermore, with the disk design it is impossible to suck the completed crystals back into the syringe for storage. It was therefore decided to change the design to an unobstructed tip to allow crystal storage and to minimize nucleation.

The results of the KC-135 experiments gave the investigators assurance that when these systems were flown in the space shuttle, the drops would survive the moderate shocks encountered. The protein crystal growth experiment was successfully flown on four different shuttle missions. The porous glass disks were not used because of the previously noted need to withdraw the drops with grown crystals back into the syringes just before return to Earth's gravity. This change protected the crystals and significantly slowed the crystal growth. The results from these preliminary growth experiments show significant changes in growth patterns and will be presented in a later paper. Further ground-based and flight studies are being pursued.

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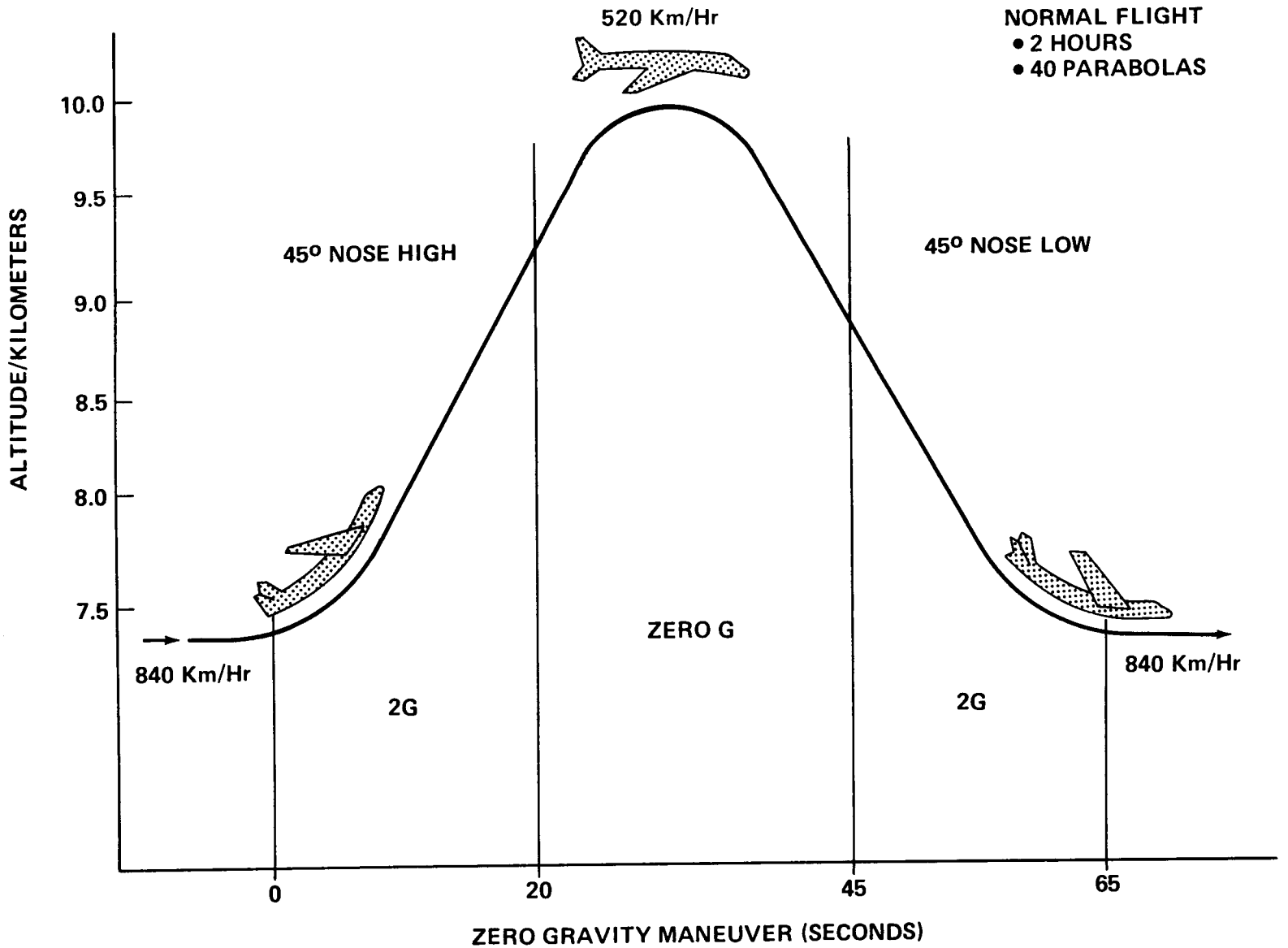

Figure 1. Flight parabola of KC-135 low-gravity simulation aircraft.

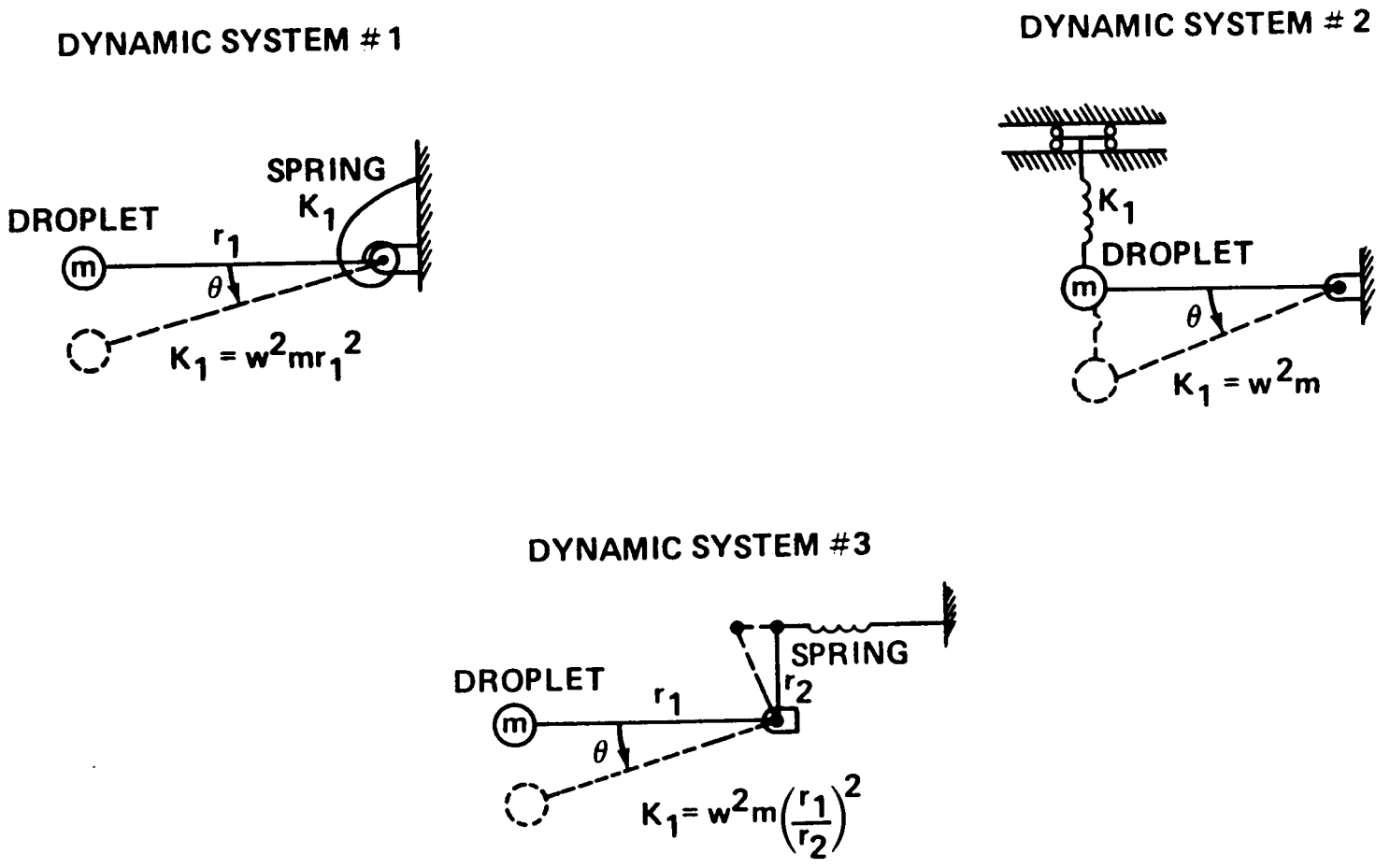

Figure 2. Model dynamic systems.

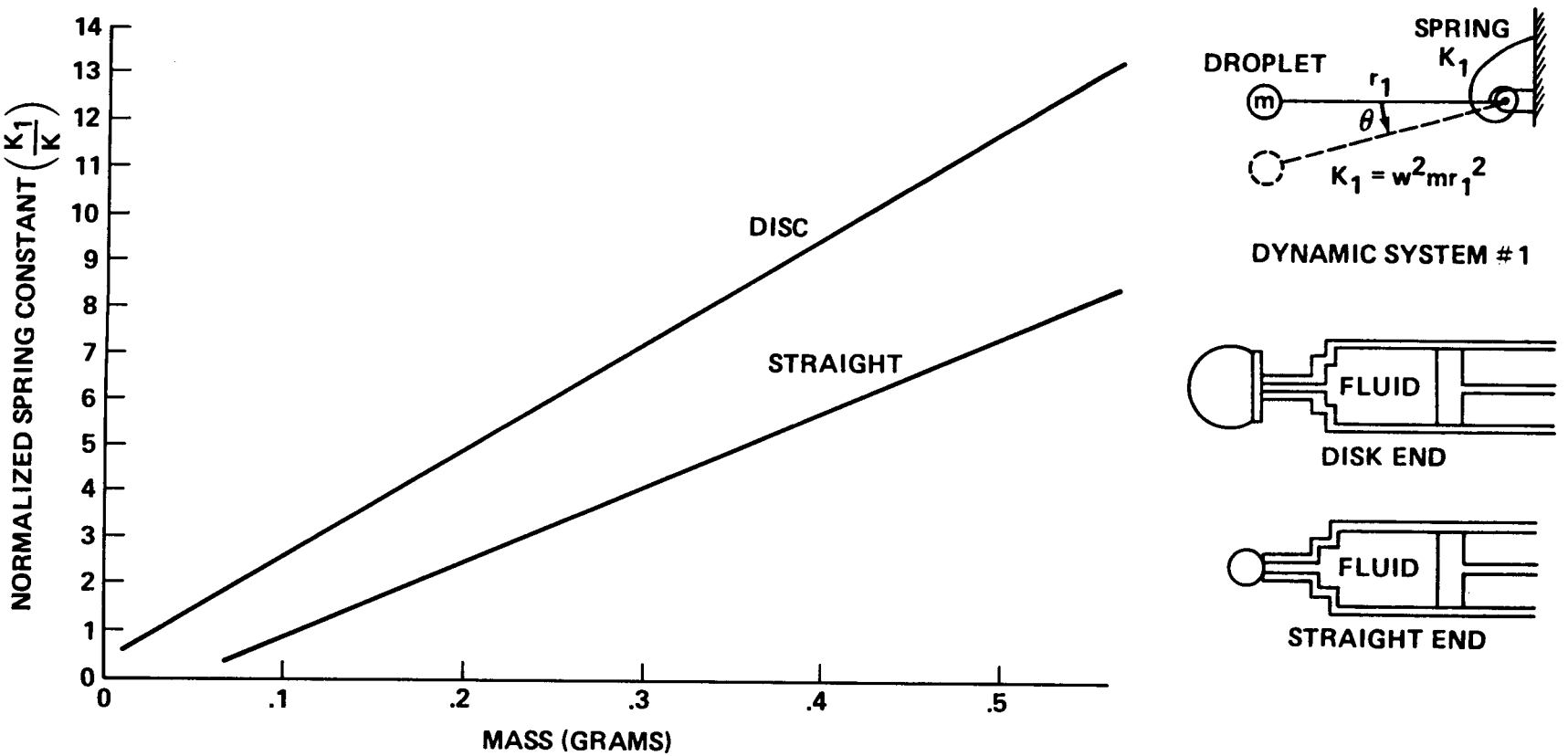

Figure 3. Normalized spring constant versus mass for model dynamic system No. 1 applied to flight data for disk and straight end syringe tips.

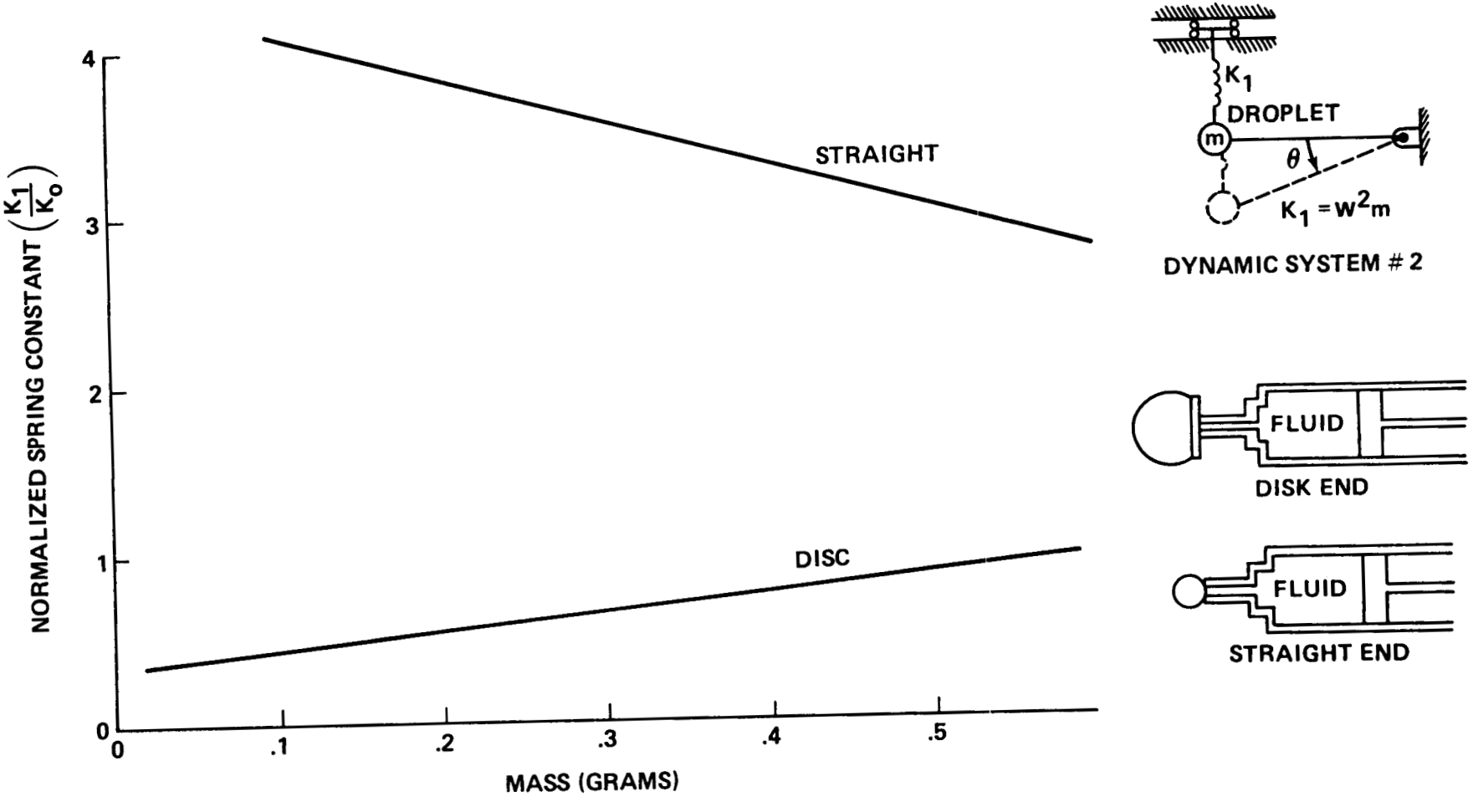

Figure 4. Normalized spring constant versus mass for model dynamic system No. 2 applied to flight data for disk and straight end syringe tips.

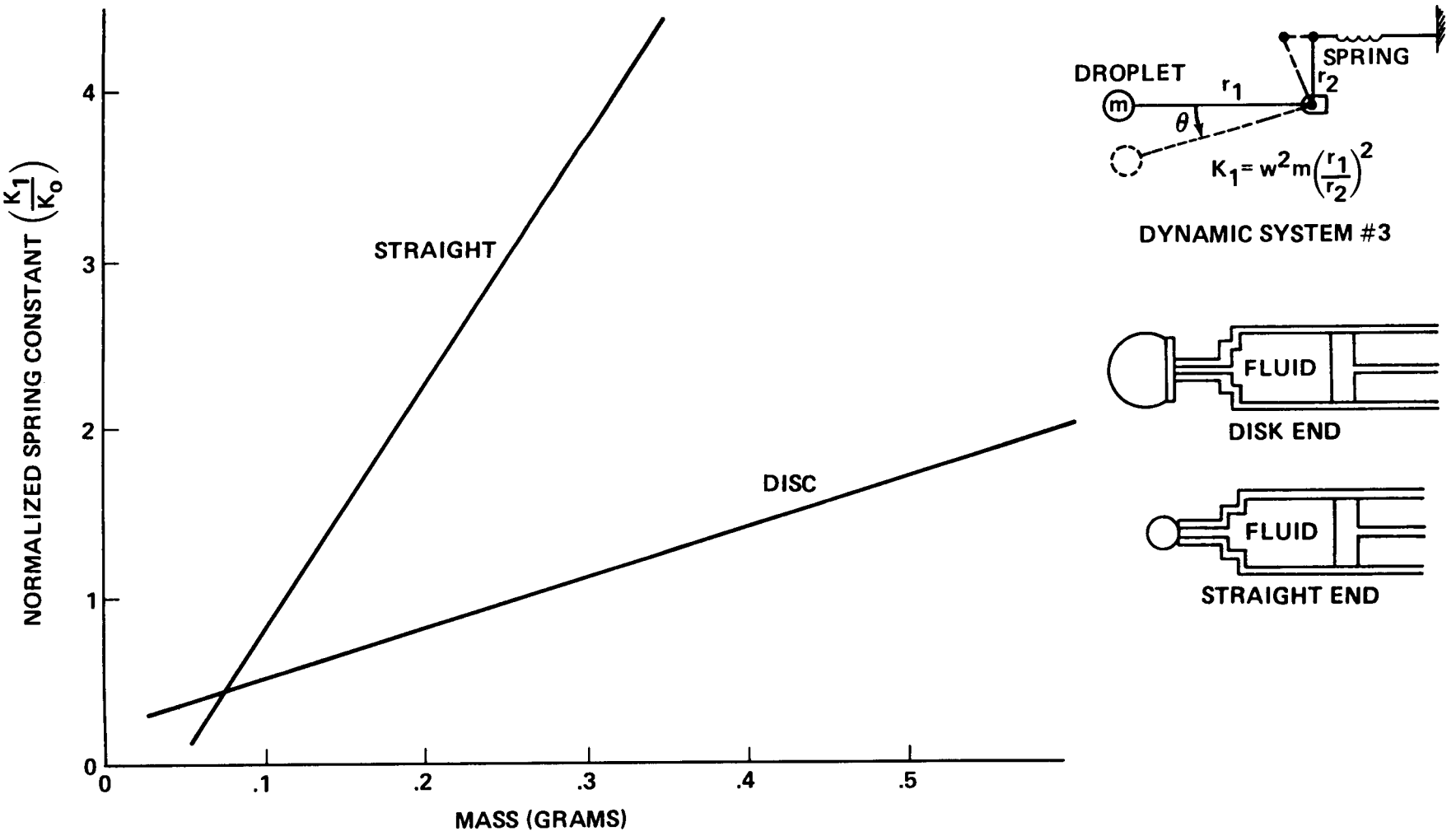

Figure 5. Normalized spring constant versus mass for model dynamic system No. 3 applied to flight data for disk and straight end syringe tips.

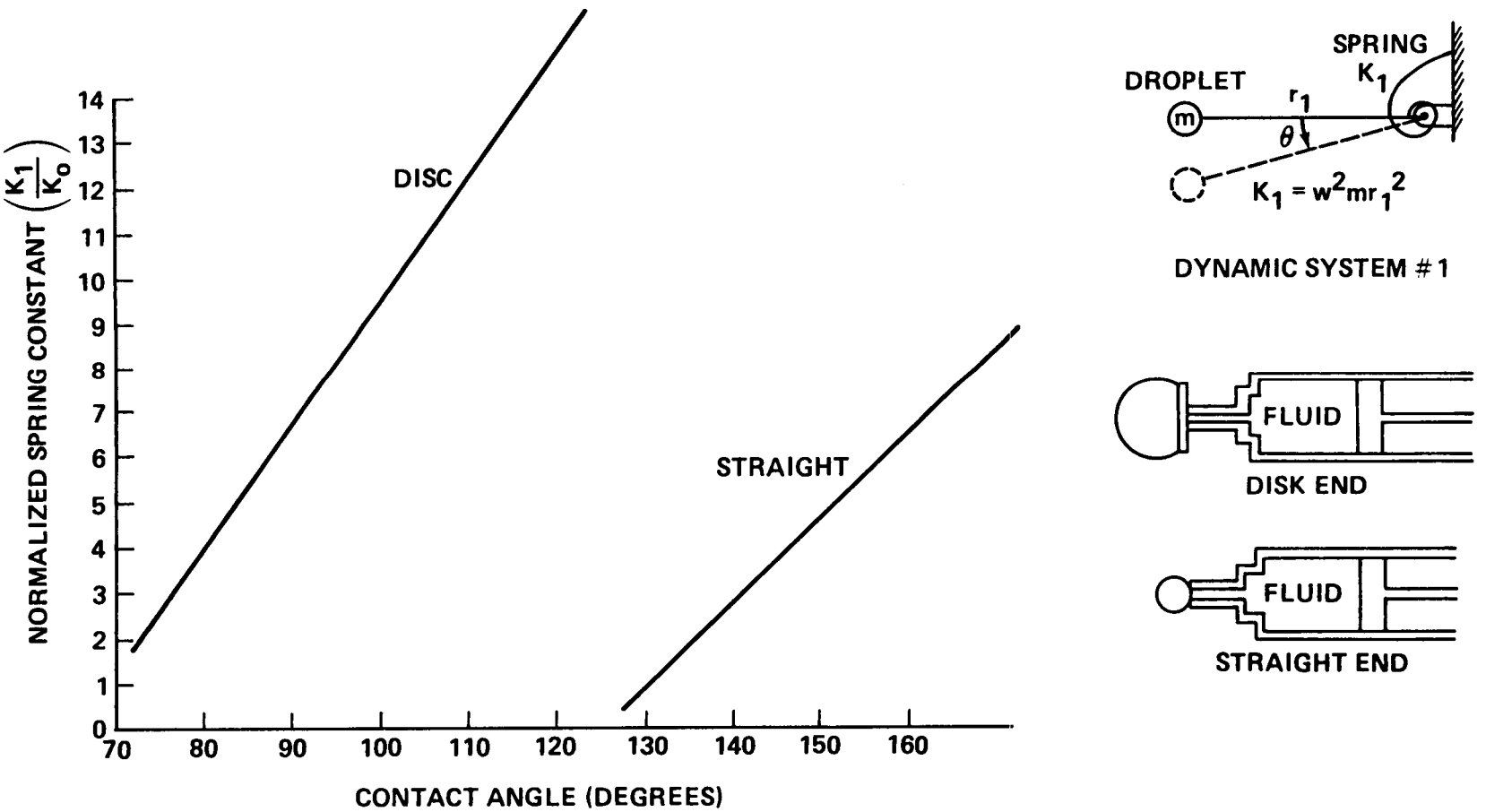

Figure 6. Normalized spring constant versus contact angle for model dynamic system No. 1 applied to flight data for disk and straight end syringe tips.

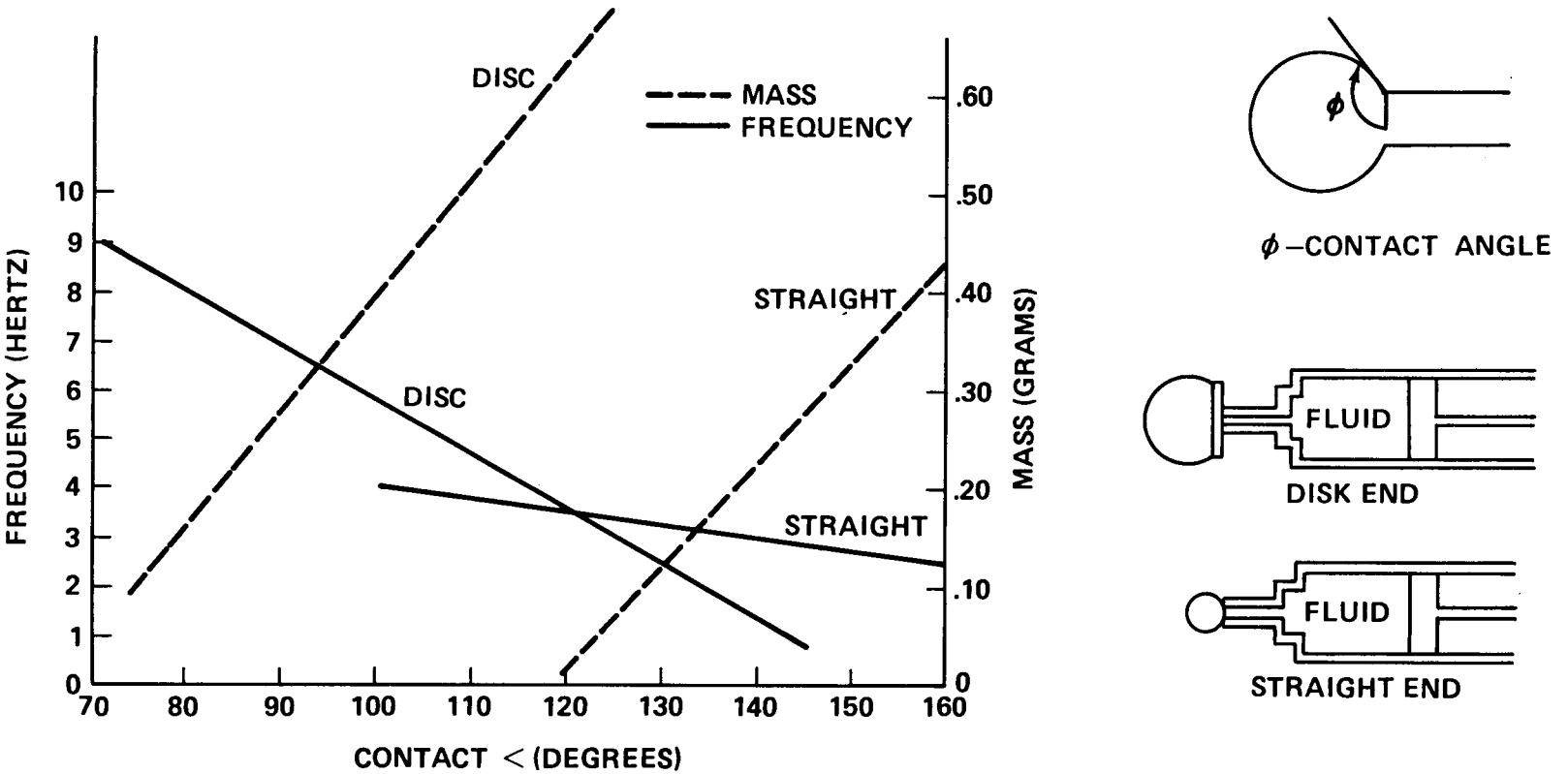

Figure 7. Vibrational frequency and mass versus contact angle.

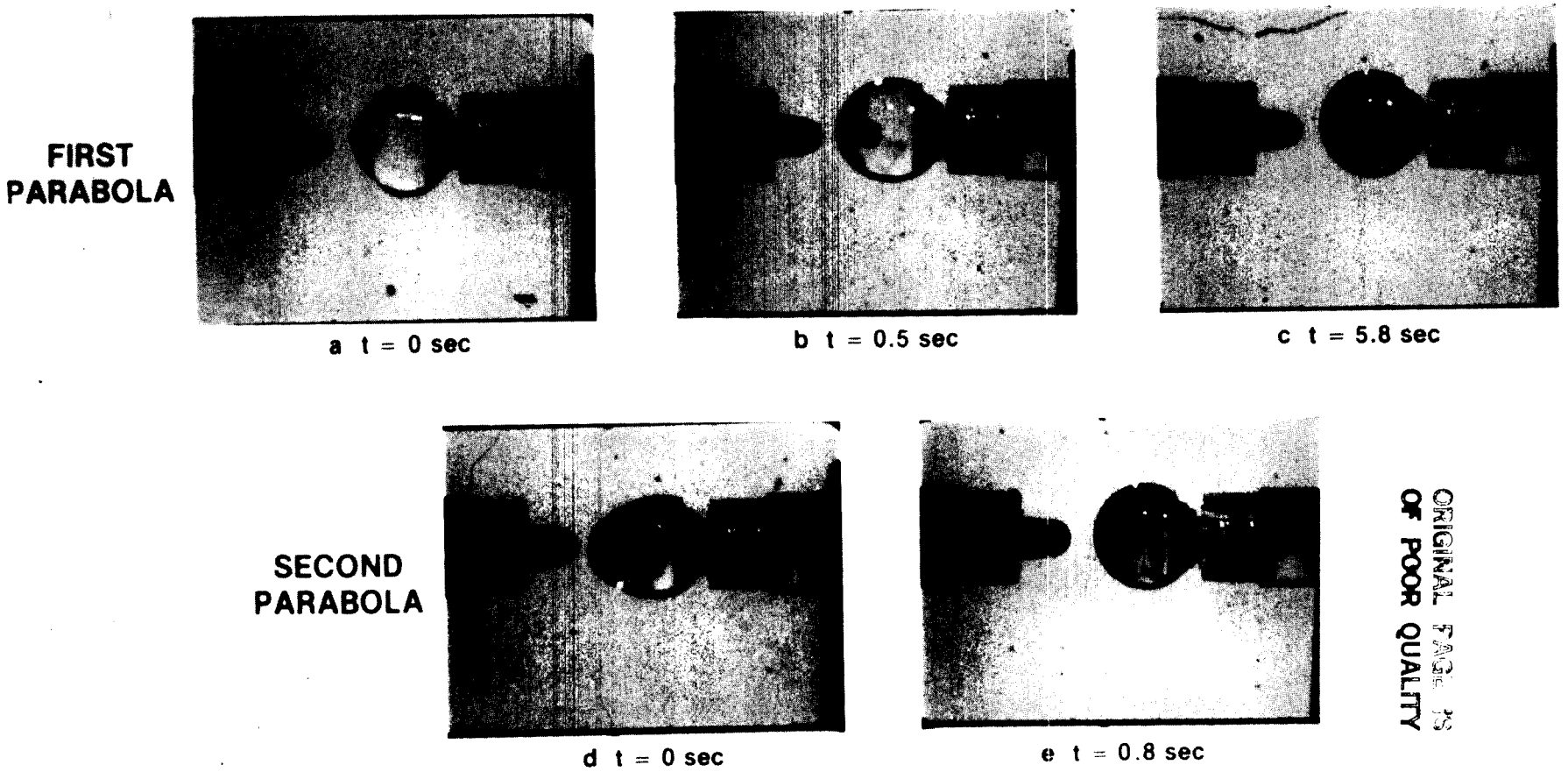

Figure 8. Stability and mixing behavior of drops during two KC-135 parabolas. Right syringe holds -0.35 ml water drop, left syringe holds dyed water.

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16. ABSTRACT

It is possible to grow protein crystals for biomedical research in microgravity by deploying a protein-rich solution from a syringe, forming a drop in which crystallization can occur with the proper degree of supersaturation. Drop stability is critical to the success of this research, due to the large drop sizes which can be achieved in space. In order to determine the type of syringe tips most suitable to support these large drops, tests were performed during brief periods of weightlessness onboard the NASA KC-135 low-gravity simulation aircraft. The drops were analyzed using three simple computer models in which the samples were approximated by modified pendulum and spring systems. It was concluded that the higher frequency systems were the most stable, indicating that of the syringes utilized, a disk-shaped configuration provided the most stable environment for low-gravity protein crystal growth.

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