Introduction

This project involves investigation of demixing of immiscible polymers in low-G and applications of this knowledge to: (a) providing a better understanding of the role of phase segregation in determining the properties of polymer blends made from immiscible polymers, and (b) purification of biological materials by partitioning between the two liquid phases formed by solution of the polymers polyethylene glycol (PEG) and dextran in water.

It should be noted that this project has changed directions again in that NASA required Celanese to drop out of the Consortium after they were purchased by a German firm.

Work has proceeded in four areas during the past year:

1. Testing of New Apparatus for Space Flight
2. Extension of Affinity Phase Partitioning
3. Refinement of Polymer Chemistry
4. Demixing of Isopycnic Polymer Phases in One-G

Testing of New Apparatus for Space Flight

F. C. Wessling has worked with us on a redesign of the low-G, phase-demixing apparatus. The new apparatus, which has been constructed, consists of an alumi-
num block containing two rows of six cavities (for a total of twelve chambers). Each cavity will contain a glass cube (1.6 cm³) which in turn will contain a polymer two-phase system and a small stirring bar. Stirring is provided by small stirring motors with magnets attached to the shafts. Thus there are six motors along the top of the apparatus and six along the bottom. In our previous design, stirring was done by passing a shaft and paddle through an O-ring into the phase system container. By eliminating the O-ring and sealing the container we should now have a much improved device; leakage and shaft freezing, which were problems with the old design, are now eliminated. In addition, the new apparatus has heating wires attached to provide heating (necessary in the GAS can) and temperature control. We have also purchased a Nikon F3 to be used with this apparatus.

The apparatus has been flown twice on KC 135 flights, and it performed well. We are now making slight modifications for automated flight as a GAS experiment. We are considering modifications to make the apparatus suitable for sounding-rocket flight.

Extension of Affinity Phase Partitioning

A major interest of ours is in developing the technique of affinity phase partitioning (with polymer two-phase systems) for eventual use in low-G separation of cells of commercial importance. Previous work has shown that gravity-driven demixing of the polymer two-phase systems greatly reduces the efficiency of phase partitioning on Earth; if this randomizing force were removed from affinity phase partitioning, a cell separation procedure of perfect selectivity (no undesired cells would be retained) would be provided. Affinity phase partitioning, previously developed by us, utilizes an affinity ligand (an
antibody) attached to PEG to pull cells into the PEG-rich phase (unwanted cells remain at the interface between the PEG-rich and dextran-rich phases).

Previously, we have applied this technique to purifying red blood cells using antibodies to the cells as affinity ligands. In the present project we have worked on extending this technique by covalently binding protein-A, rather than antibody, to PEG. Protein-A is of interest because it binds antibodies. Thus if protein-A is covalently attached to PEG, exposure to any antibody will provide ready binding, via to the protein-A, of the antibody to PEG. This would greatly simplify the technique since it would remove the necessity of covalently binding every desired antibody to PEG; a single PEG-protein-A preparation could be used for a large number of affinity separations.

We have now covalently coupled PEG to protein-A and have shown that this material will bind to the antibody to human red blood cells and this complex will pull these cells into the top PEG-rich phase. Thus we have greatly simplified the general application of the phase partitioning technique to cell separations.

Work in the past quarter has dealt with application of this technique to purifying megakaryocytes. These are cells which are of interest for future space experiments to test the limits of the technique on large cells for which gravity-driven sedimentation of the cells themselves is a problem. Preliminary results are promising.

Refinement of Polymer Chemistry

For the affinity partitioning procedure it is necessary that we have effective chemistry for coupling PEG to proteins. An additional chemistry requirement is for coupling PEG and dextran to glass to control phase demixing.
(discussed below). Thus we have an on-going effort in development of the required polymer chemistry.

1. PEG-Protein Chemistry

We have had a long-term effort in evaluating and developing the chemistry for attaching PEG to proteins. A large segment of this work is now approaching completion. In the last quarter we have completed the bulk of the work necessary to: (a) show the relationship between coupling method and protein activity; (b) understand the effect of organic solvents on protein activity and stability; and (c) show the effect of linking proteins to solid supports (glass). We are now writing up this work.

2. Improvement of Coating Chemistry for Control of Wall Wetting

A key part of this research program lies in controlling the demixing rate of immiscible polymers by controlling wall wetting by the immiscible phases. Thus we have been examining the effect of covalently bound dextran on the demixing rate for the immiscible phases formed from solution in water of dextran and PEG. It is obviously critical for this work that the chemistry used to bind dextran to the glass containers be effective. In the past we have evaluated our chemistry by wet chemical methods. A great improvement in this evaluation procedure results from use of X-ray photoelectron spectroscopy (XPS or ESCA). With this technique it is possible to directly determine the elemental composition of the surface with great accuracy.
Our method for coupling dextran to surfaces has been to first apply an amine sublayer and then to couple the dextran reducing terminus to this amine group. During the past quarter we have used XPS to greatly improve (factor of three) the thickness of the amine sublayer. Basically this improvement came from leaving the amine reagents on the glass and heating at 120°C. Previously we had been washing the reagents off before heating under the supposition that the reagents were held by hydrogen bonding (as published in the literature).

In the next quarter we will apply the XPS technique to evaluate coupling of dextran to the amine sublayer.

**Demixing of Isopycnic Polymer Phases in One-G**

We have been making a major effort to better understand the gravitational inputs to phase demixing. The question here is: what are the forces which lead to separation or demixing of two stirred but immiscible liquids in the absence of gravity-driven sedimentation? To advance work in this area we will quantify the phase demixing of isopycnic phase systems. The apparatus for studying this process has been assembled (consisting of a Nikon F3 with motor drive and data back, and a 2 in. by 2 in. thermostatted cell to contain cuvettes holding two-phase systems). We have obtained quality photographs of the phase demixing process as a function of time. Now we are working on automation of the characterization process (i.e., determination of the growth rate of droplets or domains) by use of an Omnicon Scanner. In addition we are examining the effect of polymeric wall coatings (dextran in particular) on the rate of demixing; the goal is to control the rate and to control which phase (dextran-rich or PEG-rich) goes to the center of the cuvette. Coating effects are observed, but it would be premature at this point to attempt quantitative description of these effects.
Contact-angle measurements are also being done, as part of another project, to aid in understanding the coating effects. These studies indicate that a dextran wall coating will lead to the dextran-rich phase against the wall of the cuvette.