FINA REPORT NASA-36955 D. O. 100 FROM NASA/MSFC

FNAS PHASE PARTITIONS

JAMES M. VAN ALSTINE, PH. D.
ASSOCIATE PROFESSOR
UNIVERSITY OF ALABAMA IN HUNTSVILLE

Table of Contents

1. Introduction and Overview
2. Summary of Results
3. Future Areas of Research
4. Publications Patents and Training
5. Support of NASA-Related Research

Appendices

1. List of Publications
2. Results from Previous NASA Grant
3. Methods to Mix Fluids in Low G - Summary
4. Partition Column Chromatography - Abstract
5. Electrokinetic Characterization of Hydrophilic Polymer Coatings - Manuscript
6. P.I. Resume
1. Introduction and Overview

Project NAS8-36955 D.O. #100 initially involved the following tasks:

a) Evaluation of various coatings' ability to control wall wetting and surface zeta potential expression

b) Testing various methods to mix and control the demixing of phase systems

c) Videomicroscopic investigation of cell partition.

Three complementary areas were identified for modification and extension of the original contact (to 5/10/93). They were:

d) Identification of new supports for column cell partition

e) Electrokinetic detection of protein adsorption

f) Emulsion studies related to bioseparations.

Tasks c and f were dependent on MSFC procurement of a video image analysis system which was contracted by NASA to Thomas Optical Measurement Systems of Columbus Georgia (phone 706-571-9373). Inspite of a contract being let some years ago by MSFC (see R. Cronise, ES76, phone 544-5493) ) only part of the system was ever delivered to UAH. This severly compromised the work that was accomplished on tasks c and f, and reduced the amount of data that could be gathered (by eye) on task a. However a reasonable amount of work (concept proof and preliminary experimental studies) were accomplished on tasks c and f. This work was sufficient to produce a video (previously supplied to P. Rhodes ES76) and successfully support a CDDF sponsored investigation within ES76. In compensation for the work which could not be performed on tasks c and f, extra work was accomplished on task a. Tasks b, d and e being completed in the manner originally envisioned (see below).

The most successful accomplishments involved coating technology. The coatings developed and electrokinetic evaluation methodology developed led to direct support of work by P. Rhodes et al. in MSFC ES76. At this time a small ($30K) follow on contract (NAG8-955) has been let. The tasks associated with this contract include:

a) Further development of long lasting, easily applied, coatings to control electroosmosis.
The research done in regard to mixing phase systems in low gravity is summarized in the table in Appendix 3. In regard to its general applicability to bioprocessing, and other experiments involving the handling and mixing of aqueous phases in low gravity, this technology found specific applications in regard to low gravity experiments recently conducted onboard both KC-135 (6) and Space Shuttle craft (see Sections 3 and 5).

Coatings developed in association with the present grant have been shown to control phase wall wetting (1) and protein adsorption (5). The significance of these developments, in regard to materials processing in space, are discussed in Section 3. The coatings technology developed and improved in regard to electroosmosis studies can also be applied to partition column chromatography (PCC). An example of the power of PCC is shown in the Meeting Abstract presented in Appendix 4. This application is being pursued with R. Cronise of MSFC/ES76. Mr. Cronise has successfully developed coated PCC support materials whose wetting performance is superior to commercial supports currently being marked by a major pharmaceutical firm.

The chemistry used to produce polymer coatings to control electroosmosis has also been applied to the development of affinity partitioning (2) as well as the development, and recent patenting by MSFC, of Polymer Affinity Electrophoresis. Work in this area includes practical Immunoaffinity Electrophoresis of Cells - a target of research by many scientists for the last seventy years.

UAH is now in position to test the above technology to various biomedical applications. Nonfinancial, informal technological exchange is being initiated with a number of companies (Supelco, Dionex, Coulter, and IDC) interested in testing polymeric derivatives in a variety of commercial apparatus, including capillary electrophoresis apparatus, and automated particle electrophoresis apparatus. The results of this collaboration have already allowed us to develop coatings which enhance the performance of the DELSA 440 commercial electrophoresis apparatus via control of electroosmosis plus wall adsorption of solutes or air bubbles (7).

3. Future Areas of Research

Four new areas have been identified for cooperative investigation with MSFC-related investigators. The first involves development and use of coatings that control electroosmosis and nucleic acid adsorption (in 1 mM buffer). Recent experiments (conducted in ES76 with P. Rhodes) have shown that such coatings allow more effective (direct) visualization, and hence modeling, of the free solution electrophoretic behavior of fluorescently labeled DNA molecules. The
second project area (also with P Rhodes) involves use of coatings to probe molecular mechanism(s) to explain the utility of externally-applied electric fields in capillary zone electrophoresis (CZE). Use of such fields is a "hot" topic in CZE as they appear to improve resolution by an unknown mechanism. The favored hypothesis, that they function by altering capillary surface charge, can be directly verified via capillaries chemically modified to exhibit different surface charge characteristics. In addition the use of such coatings should reduce protein adsorption (and crystal growth) on optical and other chamber surfaces. The above two areas have been preliminary funded for investigation under grant NAG8-955. The other two areas will be proposed for future funding and involve protein crystal growth (PCG).

NASA supports and undertakes research related to identifying and exploiting the low gravity (and other unique conditions) of space craft. In the field of biotechnology these experiments are typically in the areas of macromolecular crystal growth (e.g., protein crystal growth) and separation science (e.g., free solution electrophoresis of ultralarge molecules (nucleic acids, protein assemblies, etc.). In space, as on earth, these experiments are affected by interfacial factors, which can be controlled by polymer coatings. However the research of Van Alstine et al., (as exemplified by contracts NAS8-955 and NAS8-36955, D. O.100) has heretofore been limited to separation science.

Protein and other forms of macromolecular crystal growth are typically undertaken in chambers in which a droplet of solution containing the macromolecules of interest is "suspended" from a syringe tip in one half of a chamber. Chambers are often made of glass or plexiglass so that crystal growth can be visually monitored. As the droplet looses water via evaporation (and condensation onto absorbent material in the other half of the chamber) the macromolecules precipitate as crystals. The ideal situation is to have the solute precipitate as one or more large crystals which growth free in solution. However all too often an expensive space experiment is spoiled by non-specific adsorption of protein onto chamber walls:

1. Altering wetting of the droplet to the syringe tip with resultant loss of the droplet onto the absorbent material.

2. Giving rise to numerous nucleation sites for imperfect crystals to grow attached to the wall (and dilute the soluble protein available for feeding better crystals growing in suspension).

As noted above NAS8-36955 D. O. 100 has allowed Van Alstine and MSFC collaborators to show that:
1. That nonspecific adsorption of proteins (5) and nucleic acids (as well as particles such as cells) can be greatly (e.g. 99%) reduced by coating chamber surfaces with covalently attached neutral polymers.

2. That hydrophilic polymer coatings exist which can enhance solution wetting while reducing protein adsorption (1).

3. That electrokinetic measurements can be used in conjunction with other chemical methods to develop and optimize such coatings (3-5).

One future line of research [in collaboration with Dr. A. McPherson, U. Cal. Riverside, and (hopefully) with Dr. M. Pusey of MSFC/ES76] is to develop polymer coatings to enhance existing PCG apparatus.

The second future line of research (in conjunction with Dr. M. Pusey and Dr. Nadarajah of UAH) is to investigate correlations between optimal conditions for protein crystal growth and protein crystal surface (zeta) potential in relation to protein monomer net charge.

These studies have the potential to improve NASA-related PCG studies on earth and in space. The coating technology is particularly exciting as it is a cost-effective method to improve the performance of existing hardware.

4. Publications and Training

The contract resulted in 7 publications, 9 published abstracts and 8 presentations. One NASA patent was awarded. D. O. 100 also resulted in training for two students and one junior professor. These are detailed in Appendix 1.

5. Support of NASA-Related Research

Three MSFC Co-I's (L. Karr, P. Rhodes, and M. Pusey) were initially invited to participate formally or informally in this research. L. Karr declined due to taking educational leave. M. Pusey did not participate but intends to participate (with Dr. N Nadarajah) on studies related to protein crystal zeta potential and growth (see above). Care was taken to isolate the basic science aspects of the grant from the M. S. thesis work R. Cronise (NASA/ES76), or support to UAH's CCDS from NASA Code C. The latter involved development of a multi-transfer, multi-sample countercurrent distribution apparatus for the Space Shuttle. This ORSEP (Organic Separation) apparatus flew successfully in July 1993.
The ORSEP experiment was the first demonstration of a multi-step chromatographic separation process in low gravity. All four samples flown provided interesting results and the apparatus functioned without fail. ORSEP was dependent on mixing aqueous phases in low gravity and then controlling their demixing kinetics and final disposition via the use of coating/wetting technology. ORSEP was therefore dependent on technology to control phase mixing and wetting which was first developed by Van Alstine et al. at MSFC (Appendix 2) and on results from the present proposal. Improving this technology was one of the aims of the present proposal and the success of ORSEP (which was chosen for flight long after D. O. 100 was funded) underscores the potential applications of the research conducted.
Appendix 1.

Publications and Presentations


Abstracts


Presentations


Appendix 2. Previous NASA Sponsored Research

Drs. Harris and Van Alstine were Co-Investigators on NAS8-35333 "Cell Partition in Two Polymer Phases" (Oregon Health Sciences Center - D.E. Brooks, P. I.). Research funded under this proposal was directed towards flight experiments used to test the hypothesis that aqueous polymer phase systems (APTPSs) would spontaneously demix in low gravity via unique mechanism(s) at a rate commensurate with bioprocessing. As one small part of this work we initiated the polymer coating research which is important to our present research. Experiments were flown on STS-51D, 51L (Challenger) and -26. In addition the research resulted in 15 publications, numerous presented abstracts, invited colloquia and two patents. What follows is a brief summary of related results and publications.

NASA's interest in phase partitioning began in the late 1970's with a proposal from D. E. Brooks of the Oregon Health Sciences Center (OHSC) to develop electrophoretic methods to control the rate of demixing and final disposition two phase systems in microgravity. Initial research supported the feasibility of this and other lines of partition research (1). Closely interacting groups at OHSC, the University of British Columbia (UBC), the University of Alabama at Huntsville (UAH) and Marshall Space Flight Center (MSFC) conducted research encompassing, theoretical and applied studies on APTPSs, as well as novel applications of partitioning and related technologies. In order to achieve the prime goal of evaluating the effect of gravity on cell partitions obtained on Earth, and carry out unique bioseparations in space specific tasks needed to be accomplished:

1. Development of control "isopycnic" phase systems possessing phases with equal densities and use of such systems to evaluate the ability of APTPS emulsions to demix spontaneously in space (2).

2. Development of specific hardware and experiments for KC-135 aircraft, Get-a-way special shuttle experiments, and sounding rocket flights (2,3).

3. Analysis and understanding of phase system physical properties such as interfacial tension, viscosity, and electrostatic phase potentials, as related to system composition (polymer and buffer salt type and concentration) (4-6).
4. Methodology to record and analyze the demixing characteristics of high (i.e. 50%) volume fraction, aqueous polymer phase systems in chambers holding 1 to 5 ml samples (above and 2).

5. Development of materials and coatings capable of differentially interacting with the phases in order to "passively" control the demixing and final disposition of the phase systems (7-11).

6. Understanding the effect of chamber shape on the demixing kinetics and final disposition of the phases (8,12).

7. Development and testing of magnetic, electrophoretic, and other methods to "actively" control the demixing and final disposition of the phases (1,4,12).

8. Understanding the effect of phase system volume ratios and physical properties with regard to points #5, #6 and #7 (3,8,12).

9. Integrate the above to develop automated hardware capable of efficiently carrying out multisample, multistep partition separations in unit-g and low-g (1,12).

10. Development of partition methods separate specific cell populations on the basis of antigenic and physiologically important cell surface characteristics (13-15).

11. Definition of particle separation challenges of medical and biotechnical importance amenable to #10 above and the unique facility/time constraints of space bioprocessing (11,15,16)).

12. Development of a theoretical understanding of the forces influencing phase demixing, and the separations obtained via partitioning, on Earth and in space.(2,3,8,9).

These tasks have all been accomplished or are being actively pursued and the reader is encouraged to consult the publications referenced (for a review see 12 which is Appendixed). It should be noted that much of the work is of practical consequence for other separation methods and the present grant (NAS8-36955 D. O. 100). such as electrophoresis (4,9,11,12) and chromatography (17,18). For example polymeric coatings developed to control phase wall wetting in low g also make excellent "tethers" for anchoring proteins to
surfaces without loss of protein activity due to surface interaction (19). PEG-derivatized fatty acyl molecules and immunoglobulins (antibodies) have been shown to be potentially useful for the separation of biological materials by hydrophobic affinity (20) or immunoaffinity partition (13-16). Our NASA sponsored research into the basic physical properties of polymer two phase systems (12,21,22) is used by a variety of scientists working on theoretical (23-25) as well as applied aspects of partition biotechnology (18,23,26-30).

References Cited


APPENDIX 3.

SUMMARY OF EXPERIMENTS CONCERNING THE MIXING OF PHASES IN LOW GRAVITY

Table 1 summarizes the results of the mixing/demixing experiments conducted under this task and related investigations. In general applications involving low gravity experiments and materials processing will have to be done via methods that are under direct control of the experimenter. This narrows the methods down to manual shaking by an astronaut or electronic methods. Electronic methods (including acoustical methods) are ideally suited to automated control via computer, electronic timer or tele-science interfaces. Heating effects, deadspace/trapped volume effects, phase wetting effects and mechanical seal (leak) problems act to limit the exact applications any method can be applied to. One also has to consider noise levels, power consumption, safety (including materials safety documentation of components) and issues associated with potential malfunction. At this time magnetic mixing, cosolute heating, and nutational pump mixing appear to offer many advantages as do acoustical methods. Choice of method will be influenced by the exact application but should include preliminary studies (undertaken in chambers similar to those to be used in low g with, if possible, air-bubble free, density matched phases.

For ORSEP on Spacehab 01 stir-bar mixing was chosen to mix 1.5 ml cylindrical chambers of 10 mm diameter. The stirrers were 3 mm diam x 8 mm long axis dimension and were coated to control phase wetting (which could not be accommodated with teflon coatings). The sequence followed involved a slow ramp up (0 to 600 rpm) mixing over 15 seconds followed by 60 seconds of non-mixing during which the stirrer re-aligned itself with the field. This sequence was repeated six times. It should be noted that the glass coatings developed in the present proposal could be used to control the wetting of magnetic stirrers coated with glass. However on ORSEP gold-coating was used due to its superior ability to resist mechanical or chemical ablation.
<table>
<thead>
<tr>
<th>Method</th>
<th>Mixing</th>
<th>Energy Usage</th>
<th>Reproducability</th>
<th>Low-η Effectiveness³</th>
<th>Organic/Water</th>
<th>Mechanical Complexity⁴</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Pump</td>
<td>Shear</td>
<td>Moderate</td>
<td>Very Good</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>- Dead space in pump chamber and lines</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Possible excessive shearing of biologicals (controllable)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Possible heating effects (unlikely)</td>
</tr>
<tr>
<td>2 Plunger</td>
<td>Shear</td>
<td>Moderate</td>
<td>Moderate</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>- Mechanically complex</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Mixing effectiveness questionable</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Complex to control electronically</td>
</tr>
<tr>
<td>3 Automatic Shaking</td>
<td>Agitation</td>
<td>Moderate</td>
<td>Good</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>- Only one motor unit required</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Requires movable frame for plates</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Space usage requires mixing ball in chambers (see row 8)</td>
</tr>
<tr>
<td>4 Acoustical Agitation (and heating)</td>
<td></td>
<td>High</td>
<td>Moderate</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>- May require mixing ball in chambers (see row 8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- High energy requirements</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Heating may complicate (see row 4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Best for small volumes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Possible effects on biologicals</td>
</tr>
<tr>
<td>5 Heating</td>
<td>Thermal</td>
<td>High</td>
<td>Moderate</td>
<td>+++</td>
<td>N.A.</td>
<td>++</td>
<td>- Variable system properties and surface tension effects</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Heating and cooling may need fine control and monitoring</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Long time periods required for each step</td>
</tr>
<tr>
<td>6 Magnetic Stir-Bar</td>
<td>Shear</td>
<td>Moderate</td>
<td>Good</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>- Stir-bar in chamber has same drawbacks as mixing ball, except held in place (see row 8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Can use propeller in each half of chamber</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Seal integrity may be a problem</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Can take advantage of blade wetting by phases</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Poor efficiency with high tension systems</td>
</tr>
<tr>
<td>7 Propeller</td>
<td>Shear</td>
<td>Moderate</td>
<td>Good</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>- Requires crew time</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Less effective with high tension systems</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Variable mixing due to operator characteristics</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Requires use of mixing ball in chambers with complications due to ball residual motions, phase wetting, photography/spectroscopy interference</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Complicates observing initial stages of demixing</td>
</tr>
<tr>
<td>8 Manual Shaking</td>
<td>Agitation</td>
<td>H.A.</td>
<td>Good</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>- Requires crew time</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Less effective with high tension systems</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Variable mixing due to operator characteristics</td>
</tr>
</tbody>
</table>

Notes:

1 Relative comparison. Manual mixing shown in row 8 for comparison. Other methods electrically driven and controlled.
2 Includes ability to vary mixing in a controlled manner.
3 Aqueous polymer two-phase systems (APTFS's) (low L-L. interfacial tension, high viscosity) and organic/water two-phase systems (high tension, varied viscosity).
4 Includes possibility of malfunctioning.
5 USRA/NASA investigations (J.W.A. et al.)
6 UAH investigations (J.M. Harris et al.)
Appendix 4: Abstract submitted to 8th Int'l Partition Meeting
New Supports for Partition Column Chromatography

Till Rosenberger¹,², Raymond C. Cronise² and James M. Van Alstine³
¹Universities’ Space Research Association
²Biophysics Branch, ES76, NASA/Marshall Space Flight Center, AL. USA 35812
³Department of Chemistry, University of Alabama, Huntsville, AL. USA 35899

Multi-step phase partition separations accomplished using Countercurrent Distribution (CCD) or Coil Centrifuge Chromatography (CCC) are limited in a number of ways, including the need for dedicated equipment, and various problems associated with scale-up. They may also be associated with drawbacks related to gravity (or centripetal force) driven particle sedimentation and fluid convection. Partition Column Chromatography (PCC) involves the use of chromatographic support material which preferentially wets and localizes one (stationary) phase as a non-wetting, complementary phase is pumped through a column. PCC significantly reduces sedimentation and convection in its employ of standard chromatographic equipment which provide maximal control of process conditions and easily scaled methodology (1). Previous research directly compared CCC with CCD (2). Current research is aimed at developing improved PCC support material and directly comparing PCC with CCD. These experiments involve the subfractionation of protein or cell mixtures and employ both commercially available (3) and custom produced PCC support material. The latter include chromatography beads covalently modified with polymer coatings originally developed to control chamber wall wetting in low gravity phase demixing experiments.

1. Müller, W., Liquid-Liquid Partition Chromatography of Biopolymers
   E. Merck, Darmstadt, 1988, p. 60.
2 Van Alstine, J. M., Snyder, R. S., Karr, L. J., and Harris, J. M.
3. Lipargel supports obtained from E. Merck, Darmstadt.

Figure 1  Comparison of 10.5 hr, 60 transfer CCD (30 s mix, 10 min settle)
and 2.5 hr PCC (Lipargel 650, 0.3 ml/min/cm²) of horse heart myoglobin
and horseradish peroxidase in a dextran T40, PEG 6000 system.
Electrokinetic Characterization of Hydrophilic Polymer Coatings of Biotechnical Significance

JAMES M. VAN ALSTINE *1, NORMAN L. BURNS 2, JENNIFER A. RIGGS 2
KRISTER HOLMBERG 3 AND J. MILTON HARRIS 2

Departments of 1Biology and 2Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899
3Institute for Surface Chemistry, P.O. Box 5607, S-114 86 Stockholm, Sweden

ABSTRACT

Analytical microparticle electrophoresis was used to characterize various polymer coatings known to control protein adsorption and related phenomena of biotechnical significance. The electrophoretic mobility of polystyrene latex microspheres and the electroosmosis associated with quartz capillaries were characterized over the pH range 2 to 11. Such characterization provides information related to surface modification. Aminopropylsilane and mercaptopropylsilane were shown to be effective sublayers for covalent attachment of hydrophilic polymers to quartz glass surfaces. Polyethyleneimine was similarly verified as an effective sublayer for polystyrene latex. Polymer coatings based on poly(ethylene glycol) and three polysaccharides, dextran, ethyl(hydroxyethyl)cellulose, and hydroxypropylcellulose, were found to significantly reduce capillary electroosmosis and microsphere electrophoretic mobility over a broad pH range. This reduction corresponds with the ability of these coatings to reduce protein adsorption and control surface wetting by aqueous polymer two-phase systems.

Introduction

Modification of surfaces with hydrophilic polymer coatings is a promising area of biotechnical and medical research [1,2]. Various polymers, including poly(ethylene glycol) (PEG), and polysaccharides such as the polyglucose dextran and ethyl(hydroxyethyl)cellulose (EHEC) effectively reduce protein and particle adsorption when coated onto biotechnically important surfaces. Such coatings function to enhance biocompatability [1,2], reduce adsorption-related band spreading and sample loss in chromatography [3,4], control adsorption-related artifacts in biochemical assays involving microspheres [5] and microtitre

*To whom correspondence should be addressed at The Department of Chemistry
University of Alabama in Huntsville, Huntsville, Alabama, USA 35899

Key Words: adsorption, coating, dextran, electroosmosis, electrophoresis, PEG, poly(ethylene) glycol, polymer, polysaccharide, protein, silane
James M. Van Alstine, Ph. D.

Department of Chemistry, University of Alabama in Huntsville
Huntsville, MSB-C203, Alabama 35899
phone 205-895-6253, fax 205-895-6349

Education

Univ. of British Columbia, Vancouver, B. S., 1975, Biochem./Chem.
Univ. of British Columbia, Vancouver, Res. Asst., 1977, Chemistry
Univ. of British Columbia, Vancouver, Ph. D., 1984, Pathology

Professional and Postdoctoral Experience

1990 - present, Associate Professor Chemistry, and Materials Science
University of Alabama in Huntsville (UAH)
1991 - present, Associate Scientist, Center for Cell Research, Penn State University
1990 - present, Director USRA NASA/MSFC Program in Microgravity Science
1993 - present, Adjunct Associate Professor of Biology, UAH
1987 - 1990, USRA Staff Scientist, NASA/MSFC, Biophysics Branch
1986 - 1987, USRA Postdoctoral Fellow, NASA/MSFC, Biophysics Branch
(USRA is the Universities Space Research Association which was formed under
the auspices of the National Academy of Sciences in 1968. MSFC is the Marshall
Space Flight Center.)

Honors and Societies

One "NASA HQ Certificate of Appreciation", Five "NASA Certificates of Recognition"
NASA MSFC Managerial "Group Achievement Award"
B.C. Provincial Government Full Tuition Scholarship - Undergraduate Studies
Multiple Sclerosis Society Research Studentship - Graduate Studies
American Chemical Society (Chapter Treasurer) - Microbi. and Biochem. Technol.
Sigma Xi (Chapter President)
American Association for the Advancement of Science

Patents

(1) Polymer Coatings to Control Electroosmosis
    Serial No. 808,981
(2) Polymer Affinity Electrophoresis of Cells and Particles
    Serial No. 376,487
(3) Materials Exhibiting Unique Surface Properties in Biotechnical Separations
    Patent disclosed and being pursued by NASA
(4) Polymer Coatings to Enhance Electrophoresis
    Patent disclosed and being pursued by UAH
RECENT PUBLICATIONS - FROM THE PAST 5 YEARS

(From 42 publications plus 44 abstracts and 30 invited presentations)


**Students**

(1) Norman Burns (Chemistry, M. S. Student), NASA Graduate Fellowship Coatings to Control Surface Zeta Potential Expression, 1990 - present

(2) Jennifer Riggs (Chemistry, B. S. Student), Alabama Space Grant Scholarship Protein and Polymer Modification of Polystyrene Surfaces, 1991 - 1992

(3) Phillip Gibson (Biological Sciences, M. S. Student) Column Cell Separation, 1991 - 1992

(4) Till Rosenberger (Biological Sciences, B. S. Graduate) Partition Column Chromatography, 1992 - present

(5) Kazunori Emoto (Materials Science, Ph. D. Student) EPSCoR Funding Polymer Coatings To Enhance Biocompatibility, 1993-present

(6) Lynette Mays (Chemical Engineering, B. S. Graduate Student) Minority Student Researcher Program Surface Characterization of Chemically Modified Quartz
Research Accomplishments (at UBC, USRA and UAH)

Development and Characterization of Ultrapure Hydrophobic Affinity Partition Ligands
Development of Statistically Valid Partition Test for Multiple Sclerosis
Experimental Verification of Role of Interfacial Tension in Cell Partition
Discovery of Reduced LCAT Enzyme Activity in Multiple Sclerosis
New Role of Polyoxyethylene Nonionic Surfactant Headgroup MW in Determining Polymer Surfactant Critical Micelle Concentration
Development of Immunoaffinity Phase Partitioning
Development of Affinity Electrophoresis of Particles Using Polymer Ligands (Patented)
Demonstration of Ability of Phase Partition to Detect Disease Related Particle Surface Characteristics (Mouse Melanoma and Gram-Negative Bacterial Infection)
Defined Polymer Coatings to Control Surface Zeta Potential Expression (Patented)
First Demonstration of the Ability of Polymer Coatings to Control Aqueous Polymer Phase Wall Wetting on Earth and in low gravity
Application of Aqueous Polymer Phase Contact Angle Goniometry to the Characterization of Polymer Coatings Used in Biomedicine
Application of Microparticle Electrophoresis to the Characterization of Polymer Coatings Used in Biomedicine
First Demonstration of Aqueous Polymer Phase Emulsion (PPE) Demixing in Low Gravity PPE Experiments as Co-Investigator on Shuttle Missions 51D, 51G, 26, IML-1 and Canex-1 Bioseparations Experiments as Principal-Investigator on Spacehab Missions 1 and 2 Establishment of NASA Funded Biosphere Student Research Program with UAH and Huntsville Botanical Gardens
First Multistep, Low Gravity, Chromatographic Separation of Biological Materials.

Particulars of Interest

Born 2/15/53, Vancouver, Canada. Canadian Citizen
Resident Alien (Green Card) since 1986 (eligible for American Citizenship)
Unmarried, Significant Other - Dr. Kristina Köhler (Post-Doc., Chem. Eng. Dept., MIT)
Hobbies - poetry, sailing, fishing

References

Available upon request
CURRENT RESEARCH

At present my research involves polymer - surface interactions of biomedical significance. In order to eliminate the randomizing effects of sedimentation and convection some experiments are being conducted onboard NASA's Space Shuttle. The "polymers" we work with are synthetic polymers or proteins. The surfaces we work with are glass, polystyrene, or (in the future) metal, as well as the surfaces of normal and abnormal cells, bacteria, or synthetic particles. The biomedical significance of our work stems from the ability of surface localized polymers to modify, and hence control, a host of phenomena such as adsorption, partition between two aqueous polymer phases, surface wetting, surface zeta potential expression, polymer - cell, and cell - cell interactions. These phenomena influence a range of biological interactions, as well as methodologies used to separate biological compounds and cells for analytical and/or preparative purposes. The breadth of our research is controlled via well chosen experiments and quality collaborations bridging several areas of study. Collaborators include medical doctors, biotechnologists, chemical engineers, biophysicists, and chemists, from industry, academia, and government.

CURRENT RESEARCH PROJECTS

Ongoing collaborators include Dr. J. M. Harris (Chemistry, UAH), Dr. K. Chittur (Chemical Engineering, UAH), The UAH Consortium for Materials Development in Space, NSF EPSCoR and NASA/MSFC.

1. **Separation of Cells on Spacehab Missions 1 and 2**
   Co-Investigators - Center for Cell Research (W. Hymer, Penn. State)
   Interfacial Dynamics Corporation (G. V. F. Seaman)
   NASA/Code C Funded project

2. **Microvideography of Column Cell Separation**
   Co-Investigators - NASA/MSFC Funded Project

3. **Polymer Coatings to Control Electrophoretic Separations**
   Co-Investigators - Dionex Corp., Supelco Corp. and Coulter Electronics

4. **Electrokinetic Methods to Characterize Polymer Coatings**
   Co-Investigator - Surface Chemistry Institute (K. Holmberg, Stockholm)

5. **Electrokinetic Microsphere Immunoassay**
   Co-Investigators - Interfacial Dynamics Corporation
   Western Biomedical Research Laboratory

6. **Interactions of Proteins with Surfaces**
   (NSF Funded Project)

7. **Theoretical Studies Related to Modeling the above**
   Co-Investigators - Chemical Engineering (J. C. Baygents, UA, Tucson)
   Chemical Engineering (A. Nadarajah, UAH)
   Chemistry/Pathology (D. E. Brooks, UBC, Vancouver)

8. **Novel Cell Culture in Biphasic Media**
   (Funded via UAH Mini-Grant)
Phase Partition Research

James M. Van Alstine, Ph. D.
Assoc. Prof.

Dept. of Biological Sciences
University of Alabama in Huntsville
Huntsville, AL 35899

NASA/MSFC, Alabama, 35899

n/a

This report documents research efforts to date in three areas of effort, videomicroscopy, cell partition, and polymeric coatings. Recent achievements are documented on attached sheets and documents.

Polymers, Phase, Partitioning
Polyethylene glycol, Cells
Electrophoresis

Unclassified/Unlimited
However proprietary as may lead to one or more patents

n/a