Spontaneous Vesicle Formation at Lipid Bilayer Membranes

David A. Edwards,* Faith Schneck,* Ingrid Zhang,* Anthony M. J. Davis,§ Hongming Chen,§ and Robert Langer®

*Department of Chemical Engineering, The Pennsylvania State University, 204 Fenske Laboratory, University Park, Pennsylvania 16802; *Department of Mathematics, The University of Alabama, Tuscaloosa, Alabama; §Department of Chemical Engineering, Harvard-Massachusetts Institute of Technology Division of Health Sciences, and Whitaker College of Health Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, and Department of Surgery, Children's Hospital, Boston, Massachusetts 02115 USA

ABSTRACT Unilamellar vesicles are observed to form spontaneously at planar lipid bilayers agitated by exothermic chemical reactions. The membrane-binding reaction between biotin and streptavidin, two strong transmembrane neutralization reactions, and a weak neutralization reaction involving an "antacid" buffer, all lead to spontaneous vesicle formation. This formation is most dramatic when a viscosity differential exists between the two phases bounding the membrane, in which case vesicles appear exclusively in the more viscous phase. A hydrodynamic analysis explains the phenomenon in terms of a membrane flow driven by liberated reaction energy, leading to vesicle formation. These results suggest that energy liberated by intra- and extracellular chemical reactions near or at cell and internal organelle membranes can play an important role in vesicle formation, membrane agitation, or enhanced transmembrane mass transfer.

INTRODUCTION

The assembly of lipids into unilamellar vesicles suspended in an aqueous medium occurs by a variety of procedures, including sonication of an aqueous dispersion of phospholipids (Huang, 1969), detergent dialysis (Enoch and Strittmatter, 1979), and reverse-phase evaporation (Deamer and Uster, 1983). In biological systems, lipids commonly associate into spherical and nonspherical vesicles, generally by detachment of a portion of lipid bilayer from a parent plasma or organelle membrane whose configuration has been rendered unstable by some combination of chemical, electrical, mechanical, or thermal stimuli (Alberts et al., 1994). It has recently been proposed (Edwards et al., 1996) that exothermic reactions near or at cell membranes, such as those between clustered receptors localized in the plasma membrane and extracellular ligands, may be involved in the initial formation of membrane deformations that subsequently grow and break off from the plasma membrane to form unilamellar vesicles during receptor-mediated endocytosis. There are numerous other cellular examples in which energy liberated by chemical reactions might support heightened mass transfer, membrane agitation, or vesicle formation. About half of the energy derived from the intracellular oxidation of carbohydrates is not used in the formation of ATP (Alberts et al., 1994), any fraction of which might provide an energy source for vesicle formation at internal organelle or plasma membranes by a vesicle formation mechanism of this kind. The observation (Gutknecht and Tosteson, 1973) that chemical reactions involving weak acids and bases near lipid bilayers and biological membranes (Barker and Levitan, 1972) can increase rates of transmembrane mass transfer may also be due, in part, to this effect.

As the mechanism of vesicle formation at lipid bilayer membranes by chemical reactions has not been reported previously, in this study we have conducted a variety of chemical reactions at model planar bilayers to directly examine the phenomenon. Our results show that exothermic reactions at or near a lipid bilayer can lead to the formation of unilamellar vesicles and demonstrate a novel method for inducing the self-assembly of unilamellar vesicles of possibly wide application.

MATERIALS AND METHODS

Materials and solutions

1-α-Phosphatidylcholine, decane, uranyl acetate, and [3H]glycerol were purchased from Sigma Chemical Co.

Methocel solutions were prepared by mixing 25 ml distilled water containing 2% methyl cellulose (Dow Chemicals) with the following: 1) streptavidin (Gibco BRL-Life Technologies) reconstituted in 25 ml buffer [0.01 M phosphate buffer (Sigma), 0.15 M sodium chloride (Fisher), 0.05% sodium azide (Sigma)]; 2) 25 ml 0.835 M sodium hydroxide (J. T. Baker Chemicals); 3) 25 ml 2.7 M ammonia (Sigma); 4) 25 ml 39.5 mM sodium salicylate (Sigma), 0.5 mM salicylic acid (Sigma), 150 mM sodium chloride (Fisher), 150 mM sodium citrate (Sigma). Aqueous solutions were prepared with 1) 0.5 mM biotin (Sigma); 2) 0.484 M acetic acid (Sigma); 3) 0.5 M hydrochloric acid (Sigma); and 4) 0.15 M sodium phosphate (Fisher) in distilled water.

Planar bilayer formation

Lipid bilayers were formed in two ways. In the first method, a solution of 0.5% egg lecithin in decane was spread across a 1-mm-diameter hole in a 1-mm-thick teflon barrier placed between the two sides (front and rear) of a side-by-side diffusion cell and allowed to thin until the appearance of a black film (Xiang et al., 1992) within some small portion of the teflon hole. In the front solution a single reactant was dissolved in an aqueous medium,
and in the rear solution a second reactant was dissolved in an aqueous medium containing 1% methyl cellulose (yielding a viscous sol). To calibrate the technique, rates of diffusion of \(^{3}H\)glycerol were measured across the spread lecithin/decane membrane throughout the process of membrane thinning. This involved withdrawing 20-μl aliquots every 5 min from the rear solution and scintillation counting to determine the glycerol concentration. A lipid bilayer membrane was determined to have formed (after the thinning of the decane/lipid film) when the rate of glycerol diffusion across the teflon barrier assumed a constant value. This normally occurred within 10 min of insertion of the teflon barrier between the front and rear solutions. In the second method, lecithin bilayers were formed using the patch pipette method (Suarez-Isla et al., 1983). Briefly, one end of a pipette is dipped into a solution of water, reactant, and 1% methyl cellulose. The pipette is withdrawn under positive pressure so that the solution remains in the pipette. The pipette tip is next placed within an aqueous solution containing 0.5% lecithin lipids and withdrawn under positive pressure so that a lipid monolayer remains at the free surface at the end of the pipette, with nonpolar lipid tail groups pointing out of the pipette into the air. Finally, the pipette is reintroduced into a third aqueous solution containing 0.5% lipids, leading to the existence of a monolayer spread over the free surface, and a second reactant, still under positive pressure. A high electrical resistance seal (resistance ~15 kΩ-cm\(^{-2}\)), reflecting the existence of a lipid bilayer, usually forms at the end of the pipette, across or near which a chemical reaction subsequently ensues between the two reactants. Electrical resistance was measured by applying an AC current, typically 50 mV at 1 Hz, between two Ag/AgCl electrodes located in the front and rear solutions near the end of the pipette. The current was measured by an ammeter (Texas Instruments), and the resistance was calculated by Ohm’s law. The pipette was glass and possessed an inner diameter of approximately 1 mm.

Microscopy

An inverted research microscope (Olympus T041) was used to detect the presence of liposomes after chemical reactions as follows: 100-μl aliquots were withdrawn from front and rear solutions using a micropipette 2 hours after formation of the bilayer and commencement of the chemical reaction. The aliquots were placed on glass slides and examined directly under the microscope at a magnification of 80×. A transmission electron microscope (TEM) was used to examine liposome structure as follows. Electron microscope grids were covered with a thin layer of electron-transparent carbon support film. A 100-μl aliquot sample from either front or rear solutions was placed on the grid. Fifteen seconds were allowed for liposomes to settle and attach to the film. The grid was next gently rinsed with two drops of water before a single drop of 2% uranyl acetate was applied to stain the sample. The bulk of the stain was washed away with another drop of water after 15 s, and excess water was drawn off with filter paper. The grid was allowed to air dry at least 30 min before examination by a transmission microscope (JEOL 1200CX).

RESULTS

Experimental

To determine whether energy liberated by the binding of membrane receptors and ligands can lead to vesicle formation, we created lipid bilayers by the patch pipette method with streptavidin in the (methyl cellulose-containing) rear solution and biotin in the front solution. Two hours after formation of the lipid bilayer and commencement of the binding reaction between biotin and streptavidin, samples were withdrawn from front and rear solutions. No vesicles were detected in the front solution by TEM. Fig. 1 shows three characteristic TEM photographs of samples extracted from the rear (methyl cellulose containing) solution from the patch pipette experiment. Unilamellar lipid vesicles, approximately 100–200 nm in diameter, were detected by TEM on the methyl-cellulose-containing side of the bilayer (the white rims bordering the vesicles seen in Fig. 1 indicate the presence of lipid bilayers).

Similar experiments were performed with sodium salicylate and salicylic acid. The sodium salicylate reaction differed from the biotin-streptavidin reaction in that it was performed entirely in the rear solution (whereas the former reaction occurred between front and rear solutions, across the bilayer), with the front solution containing a sodium phosphate buffer. We observed unilamellar vesicles in the sodium salicylate/salicylic acid system that appeared identical to those in the biotin/streptavidin system, with vesicles

![Figure 1](image_url)
again forming only in the rear (methocel) solution. Experiments performed without methyl cellulose led to vesicles being distributed between front and rear solutions, although in fewer numbers than in the case with methyl cellulose.

To examine the effect of strength of chemical reaction on vesicle formation, two additional chemical reactions were performed involving strong acid and base reactants, with the reactants being separately located in front and rear solutions. We have studied transmembrane reactions between 1) ammonia and hydrochloric acid, and 2) sodium hydroxide and acetic acid. In both cases transmembrane reactions led to vesicle formation. Many more vesicles were formed by these strong acid-base reactions relative to the weak salicylate-salicylic acid reaction (Fig. 2). In all cases studied, vesicles were observed only in the rear (methyl cellulose-containing) solution.

All of the experiments described above were performed using the pipette method of planar bilayer formation (Suarez-Isla et al., 1983). Initial experiments were performed using the diffusion cell method (Xiang et al., 1992) as well.

**FIGURE 2** Microscopic photographs of front and rear solutions after strong and weak acid chemical reactions. (a) Front solution after a neutralization reaction between hydrochloric acid and ammonia. (b) Rear solution after a neutralization reaction between hydrochloric acid and ammonia. (c) Front solution after a reaction between salicylic acid and sodium salicylate. (d) Rear solution after a reaction between salicylic acid and sodium salicylate. The dark and light portions in each photograph correspond to the two sides of the receding three-phase contact line that demarcates the sample (dark portion), air, and the dry microscopic slide (light portion).
Although identical qualitative results were obtained with this method and the pipette method, the diffusion cell method leads to the formation of larger colloids, whose structure under TEM indicates a significant production of droplets of decane stabilized by lecithin lipid molecules (Fig. 3). This is in accord with the fact that a substantial amount of decane remains in planar membranes formed using the diffusion cell method (Xiang et al., 1992); hence this method was not extensively pursued in our investigations.

**Theoretical**

We believe the mechanism underlying the phenomenon of vesicle formation by chemical reactions involves an instantaneous diminution of the "in-plane" membrane tension $\sigma$ within a finite area of the membrane, resulting in a membrane tension gradient and membrane flow. Similar "Marangoni" flows occur at fluid interfaces, as in the case of a fluid interface heated locally, resulting in a bulge of the interface in the direction of the more viscous phase (Pimputkar and Ostrach, 1980). In the present class of membrane problems, the source of membrane tension diminution $\Delta \sigma$ is energy liberated by the exothermic chemical reaction. Instantaneous spatial fluctuations in reactant concentrations near the membrane, over the length scale of the diameter (1 mm) of the pipette tip, result in spatial variations of membrane tension, in a manner reminiscent of so-called interfacial turbulence (Hennenberg et al., 1977; Buyevich et al., 1993). The spontaneous membrane motion that ensues can be quantified by considering the start-up flow at an initially planar membrane owing to diminution of the membrane tension $\sigma$ within a finite circular portion (radius $r = \epsilon$) of the membrane. The circular domain (the shape of which is chosen for convenience) can be viewed as a domain of the lipid bilayer membrane near which the chemical reaction (e.g., between biotin and streptavidin molecules) occurs with relatively high intensity. Analysis of the quasi-steady Stokes equations, in cylindrical coordinates, with continuity across the compressible membrane of normal and tangential stress, as well as normal and tangential velocity, reveals that the normal velocity of the membrane, in the very initial

![Figure 3](image-url)
instants of deformation during which the membrane remains nearly flat, possesses the form (Appendix)

\[
v_2 = \frac{\Delta \sigma}{4 \left( \mu_+ + \mu_- \right) \left( r^2 + e^2 \right)^{1/2}}.
\]

Here, the normal membrane velocity is defined so as to be positive when pointing in the direction of the phase possessing the viscosity \( \mu_+ \), and negative in the direction of the phase possessing the viscosity \( \mu_- \).

**DISCUSSION**

The results of our study show that unilamellar vesicles form at otherwise planar lipid bilayers as a direct or indirect consequence of the energy liberated by an exothermic chemical reaction near or at the membrane. Increasing the strength of the chemical reaction appears to increase the number of vesicles formed. Eliminating the viscosity difference across the membrane removes the directionality of vesicle formation and appears to result in considerably fewer vesicles being formed.

We have theoretically analyzed this process in terms of a Marangoni-type flow (as is common at fluid interfaces), in which a local membrane tension change \( \Delta \sigma \) occurs, the magnitude of which is proportional to the liberated energy of an exothermic chemical reaction near or at the membrane. The membrane tension change \( \Delta \sigma \) drives membrane motion in the vicinity of the membrane tension inhomogeneity, suggesting a possible explanation for why the number of vesicles created in our experiments increased with increasing strength of transmembrane chemical reaction. The same reasoning explains why the bulging of a fluid interface increases with increasing interfacial temperature gradient (Pimpukkar and Ostrach, 1980). Equation 1 also shows that a membrane will deform, as a consequence of local membrane tension lowering, in the direction of that side of the membrane possessing the larger apparent viscosity, indicating why vesicles form preferentially on the side of lipid bilayers possessing a methyl cellulose viscous thickener.

Reducing the viscosity difference across the membrane leads to a diminution of the normal membrane velocity, consistent with our observation that fewer vesicles are formed when no viscosity difference exists across the lipid bilayer membranes. (This observation implicitly suggests that the lipid bilayers formed in our experiments behave as compressible membranes, as further discussed below.)

Physically, the role played by viscosity can be explained by observing that the membrane tension diminution in some portion of the membrane results in a membrane stretching motion that causes fluid to flow from the bordering phases toward the membrane, at rates that are proportional to their apparent viscosities. When the viscosity of one phase exceeds that of the other, the velocity of fluid flow on this side of the membrane is smallest, and the membrane tends to move in its direction. When no viscosity differential exists, there is no normal motion of the membrane—barring thermal or external mechanical fluctuations in membrane shape that we have not accounted for in the analysis and that obviously play a role in vesicle formation when the viscosities of the bounding phases are identical.

The arguments used here to explain vesicle formation at lipid bilayers after an exothermic chemical reaction assume the lipid membranes to be compressible. The compressibility of lipid bilayers is relatively small, however, e.g., an area element of neutrophil membrane will rupture if it is extended more than 2–3%; Sung et al. (1982). To see that this very small degree of compressibility is nevertheless sufficient to allow the membrane motion described by Eq. 1, at least for the model lipid bilayer experiments considered here, we estimate the characteristic membrane area expansion \( \Delta A/A \) that occurs in our experiments as follows. A characteristic lateral membrane velocity as a consequence of membrane tension lowering is \( \Delta \sigma (\mu_+ + \mu_-) \). Given a typical lipid bilayer membrane tension of 0.1 dyn/cm (Soltész and Hammer, 1995), and a characteristic methocel viscosity (Edwards et al., 1996) of 10 P, the characteristic membrane velocity is \( 10^{-2} \) cm/s. A typical vesicle diameter is 100 nm or \( 10^{-6} \) cm, giving a characteristic time of membrane deformation \( 10^{-9} \) to \( 10^{-7} \) s. The rate of membrane expansion \( (1/A \Delta A/\Delta t = \nabla \cdot \mathbf{v}) \) can be estimated by the characteristic membrane velocity \( (10^{-2} \) cm/s) divided by the characteristic deformation length scale (1 mm), which gives \( 10^{-1} \) s\(^{-1}\). Multiplication of the latter by the characteristic time scale of deformation gives a characteristic area expansion \( \Delta A/A \sim 10^{-5} \), which is easily achievable even for relatively incompressible lipid bilayers. It is important to note as well that the model bilayers are capable of absorbing excess lipids during vesicle formation from the periphery of the suspended planar bilayers to create new bilayer membrane, evidenced by the fact that the lipid bilayers remain intact throughout the membrane vesiculation process.

A hydrodynamic analysis has been outlined elsewhere (Edwards et al., 1996) for the nucleating motion of a cell membrane during receptor-mediated endocytosis. In this similar vesicle formation problem, the length scale of membrane deformation is the scale of a clustered receptor domain (5–10 nm)—a dimension several orders of magnitude smaller than the length scale of deformation (1 mm) imposed upon the membrane by the circumference of the pipette tip in the present experiments. The preceding arguments can be used to show that the very small length scale of deformation involved in the early stages of receptor-mediated endocytosis forces the cell membrane to behave as an essentially incompressible fluid layer (i.e., it is unable to expand to the degree \( \Delta A/A \sim 1 \) that is required by a compressive/expansive motion as described in the Appendix), with normal membrane motion inversely varying with the viscosity differential across the membrane.

An implication of this study is that energy liberated by chemical reactions near cell and internal organelle membranes can lead to deformation of the membranes and possibly vesicle formation. There are a variety of examples in
which vesicles form spontaneously at the membranes of living cells, ranging from endocytosis at plasma membranes to vesicle budding from membranes of the cis Golgi network and transitional elements of the endoplasmic reticulum (ER) during vesicle shuttling between the Golgi apparatus and the ER (Hauri and Schweizer, 1992). The energy available to support such vesicle formation might derive from binding of membrane receptors and ligands (Leckband et al., 1992), as well as from various exothermic phosphorylation reactions that occur during ATP hydrolysis. The fact that in many cellular examples involving spontaneous generation of vesicles, vesicle formation is directed toward the cytosol also suggests a possibility that the gel-like structure conferred upon the cytosol by cytoskeletal proteins might act in a manner similar to that of the methyl cellulose used here—inducing vesicle formation in the direction of the more viscous phase for the hydrodynamic reasons discussed above.

In interpreting the role played by viscosity in the experiments described above, it is important to note that methyl cellulose polymers interweave in solution to render an aqueous medium macroscopically viscous, even though the “microscale viscosity” is essentially that of water, meaning that molecules ranging from water to protein tend to diffuse at approximately the same rate as through a continuum of water. The same is true of the cell cytosol. Several experimental studies (Hou et al., 1990a,b; Luby-Phelps et al., 1988) have shown that molecules whose hydrodynamic (Stokes-Einstein) radius is $\sim 15$ nm or larger are dramatically hindered in their diffusional transport through the cell cytosol. Cytoskeletal proteins act in a manner similar to that of methyl cellulose in our systems to render the cytosol “gel-like” at a macroscale, perhaps exceeding 25 to 50 nm. Thus, the analogy between model lipid bilayer systems and cellular systems appears to be strengthened by a viscosity differential across a lipid bilayer, as originates in the presence of methyl cellulose in one of the membrane-bounding aqueous phases.

A second implication of our results is that vesicle formation by chemical reactions may play a role in the enhancement of mass transfer across membranes.

Mass transfer of salicylate has previously been studied across phosphatidylcholine bilayers using the same system as studied here, without 1% methyl cellulose (Gutknecht and Tosteson, 1973), with an aim to quantify enhanced salicylate absorption from the stomach when salicylate is delivered in a well-buffered solution. In this context, the rear solution can be seen as representing an “antacid” buffer (of pH 4.9, as might exist in the stomach), whereas the front solution mimicks a well-buffered “blood” solution of pH 7.4.

Our results for the salicylate/salicylic acid system indicate that the high rate of transmembrane salicylate transport observed by Gutknecht and Tosteson (1973) owing to the presence of a well-buffered acidic solution might be due, in part, to the creation of vesicles or membrane agitation, which may either influence or add an additional enhance-

ment mechanism to the mechanism proposed by Gutknecht and Tosteson (1973), involving diminution of a diffusion boundary layer near the membrane. This possibility is supported by the fact that agitation of phase interfaces by chemical reactions has similarly been found to lead to abnormally high interphase mass transfer (Sherwood and Wei 1957), exceeding that which can be associated with diffusion layer effects.

Finally, we have observed that exothermic chemical reactions, including those that occur between membrane receptors and their ligands and between acids and bases, can lead to the spontaneous formation of vesicles at otherwise planar lipid bilayer membranes. This method of vesicle formation may possess a variety of applications, including that of increasing mass transfer across the membranes of otherwise closed and bounded vesicles. The phenomenon may also underlie important events in the enhanced transport of molecules across biological membranes.

**APPENDIX: THE INITIAL MOTION**

In terms of cylindrical coordinates with an origin at the center of the axisymmetric disturbance, the time-dependent Stokes’ equations and membrane interface conditions can be written in the form

$$ \nabla \cdot \mathbf{v}^z = 0, \quad \rho \pm \frac{\partial \mathbf{v}^z}{\partial t} = \mu \nabla^2 \mathbf{v}^z - \nabla p^z, \quad (A1) $$

$$ \left( v_z \right)_{z=0} = \frac{\partial \eta}{\partial t}, \quad (v_i^+ - v_i^-)_{z=0} = 0, \quad (A2) $$

$$ \mu - \left( \frac{\partial v_z^+}{\partial r} + \frac{\partial v_z^-}{\partial z} \right)_{z=0} - \mu^+ \left( \frac{\partial v_z^+}{\partial r} + \frac{\partial v_z^+}{\partial z} \right)_{z=0} = \frac{\partial \sigma}{\partial r}, \quad (A3) $$

$$ (p^+ - p^-)_{z=0} = \left( \mu + \frac{\partial v_z^+}{\partial z} - \mu^+ \frac{\partial v_z^+}{\partial z} \right)_{z=0} + \sigma_0 \frac{\partial}{\partial r} \left( r \frac{\partial \eta}{\partial r} \right), \quad (A4) $$

where + and − denote, respectively, the regions $z > 0$ and $z < 0$ on either side of the undisturbed membrane, and the symbols have their usual meanings. The driving mechanism, introduced at $t = 0$, is a change in membrane tension that decays on a length scale $\epsilon = 0(10^{-1} \text{ cm})$. A suitable form for $\sigma$, having a simple Hankel transform, is

$$ \sigma = \sigma_0 - \Delta \sigma \frac{\epsilon}{(\epsilon^2 + r^2)^{1/2}} H(t), \quad (A5) $$

where $H(t)$ denotes the Heaviside unit function.

The tangential stress condition (A3) implies that the velocity scale is $\Delta \sigma (\mu_+ + \mu_-)$ and hence the time scale is $\epsilon (\mu_+ + \mu_-) / \Delta \sigma$. Typical parameter values are $\mu_+ = 10 \text{ g/cm/s}$, $\mu_- = 1 \text{ g/cm/s}$, and $\sigma_0 = 0.1 \text{ dyn/cm}$. Consequently, the quasi-steady approximation is justified in Eq. A1, and then the time dependence arises passively from the kinematic condition in Eq. A2. The development of the flow from zero initial conditions is easily handled by the use of a Laplace transform defined by

$$ \mathcal{L}(f) = \int_0^\infty f(t) e^{-\sigma t} \, dt. $$

An appropriate solution of Eq. A1 that satisfies Eq. A2 is given by
The membrane

follows that

it

\( A5 \)

Only

\( S4 \)

\( S4Vr^{-} \)

which

\( S4VZ^{-} \)

= 1214

Biophysical

\( 10^{-1} \) s.

Moreover, the membrane interface
determined;

Therefore, the plane interface

\( EAcT \)

\( EA \)

of

\( EA \)

the

membrane

surface,

\( dk, \)

Evans's

\( k \)

1.

Extraction.

Thin

It is a

\( \eta = \frac{\varepsilon \Delta \sigma (\mu_+ - \mu_-)}{2 \sigma_0 (\mu_+ + \mu_-)} \)  \small{(A7)}

The membrane displacement is then given, from Eq. A2, by

\[ \eta = \frac{\varepsilon \Delta \sigma (\mu_+ - \mu_-)}{2 \sigma_0 (\mu_+ + \mu_-)} \]

which is \( O(\varepsilon \Delta \sigma /\sigma_0) \) but grows logarithmically on a time scale of order \( 10^{-1} \) s. Moreover, the slope of the membrane surface, obtained from the

r-derivative of Eq. A6 rather than Eq. A7, is given by

\[ \frac{\partial \eta}{\partial r} = \frac{\varepsilon \Delta \sigma (\mu_+ - \mu_-)}{2 \sigma_0 (\mu_+ + \mu_-)} \left\{ \varepsilon + \left( \sigma_0 /2(\mu_+ + \mu_-) \right) \left[ r^2 + \left( \varepsilon + \left( \sigma_0 /2(\mu_+ + \mu_-) \right) \right] \frac{1}{2} \right. \]

\[ \left. + \frac{\varepsilon}{\left( r^2 + \varepsilon \right)^{1/2}} \right\} \]

which is \( O(\varepsilon \Delta \sigma /\sigma_0) \) and vanishes at \( r = 0 \). Thus the linearization of the interface conditions remains valid.

REFERENCES


