



Role of transport limitation in the resistance of *Pseudomonas aeruginosa* biofilms to ciprofloxacin and levofloxacin

by Julia Dawn Vransy

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
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Abstract:

Bacterial biofilm infections are often far less susceptible to antibiotic therapy than their planktonic, or freely suspended counterparts. Transport limitation of the antibiotic within the biofilm has been proposed as one explanation for this resistance. To explore the possibility that transport limitation contributes to a decreased antibiotic efficacy, a Comparison was made between the efficacies of two fluoroquinolone antibiotics, ciprofloxacin and levofloxacin, against freely suspended and biofilm bacteria. Transport of the antibiotics to the biofilm-substratum interface was monitored using ATR-FTIR spectroscopy techniques. Both biofilms and planktonic organisms were treated with antibiotic concentrations of 100, 250, and 500 $\mu\text{g ml}^{-1}$ for 30 min and rinsed for 1 h with fresh media. Antibiotic efficacy was determined by the ratio of culturable bacteria to total cell counts. The experimental ATR-FTIR transport data was then simulated with a mathematical computer model containing the processes of molecular diffusion, adsorption, and desorption to provide possible explanations for differences in antibiotic delivery. Levofloxacin was found to be more efficacious against planktonic organisms than biofilm cells. However, no difference in efficacy was seen when planktonic and biofilm bacteria were treated with ciprofloxacin. ATR-FTIR results showed that the biofilm provided very little transport limitation for each antibiotic, thus suggesting that the reduced susceptibility of a biofilm to antimicrobial treatment may be due to factors other than transport.

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ABSTRACT

Bacterial biofilm infections are often far less susceptible to antibiotic therapy than their planktonic, or freely suspended counterparts. Transport limitation of the antibiotic within the biofilm has been proposed as one explanation for this resistance. To explore the possibility that transport limitation contributes to a decreased antibiotic efficacy, a comparison was made between the efficacies of two fluoroquinolone antibiotics, ciprofloxacin and levofloxacin, against freely suspended and biofilm bacteria. Transport of the antibiotics to the biofilm-substratum interface was monitored using ATR-FTIR spectroscopy techniques. Both biofilms and planktonic organisms were treated with antibiotic concentrations of 100, 250, and 500 $\mu\text{g ml}^{-1}$ for 30 min and rinsed for 1 h with fresh media. Antibiotic efficacy was determined by the ratio of culturable bacteria to total cell counts. The experimental ATR-FTIR transport data was then simulated with a mathematical computer model containing the processes of molecular diffusion, adsorption, and desorption to provide possible explanations for differences in antibiotic delivery. Levofloxacin was found to be more efficacious against planktonic organisms than biofilm cells. However, no difference in efficacy was seen when planktonic and biofilm bacteria were treated with ciprofloxacin. ATR-FTIR results showed that the biofilm provided very little transport limitation for each antibiotic, thus suggesting that the reduced susceptibility of a biofilm to antimicrobial treatment may be due to factors other than transport.

INTRODUCTION

Modern medicine and engineering have together created new implantable devices for improved healthcare. Unfortunately, these medical advances are not without problems--the number one cause of medical device failure is infection. Bacteria which colonize the surfaces of implanted devices form biofilms which are less susceptible to the immune defenses of the human body and antibiotic treatments. Because of this resistance, infections can persist which will result in tissue damage, implant failure due to lack of tissue integration, and life threatening bacteremias. Often the only recourse is to remove the colonized device surgically which adds more costs and discomfort to the patient. If the mechanisms of bacterial resistance were better understood, it would be possible to design more effective antibiotics to prevent and treat device-related infections.

Two main theories have been proposed to explain the resistance of bacterial biofilms to antimicrobial agents. The first theory is based on physiological differences between attached organisms and their freely suspended counterparts while the second concept proposes that the biofilm acts as a transport barrier to the antimicrobial agent. The goal of the research presented in this thesis was to ascertain whether transport limitation of an antibiotic was responsible for its decreased efficacy. The objectives were (1) to compare the efficacies of two fluoroquinolone antibiotics against freely suspended and biofilm organisms and (2) to monitor the penetration of these two

antibiotics to the substratum of a biofilm using attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) techniques.

LITERATURE REVIEW

Biomaterial-Centered Infections

Due to the increasing use of medical devices and implants in modern medicine, much attention has been given to their associated problems. One of the greatest problems related to medical device use is infection. Biomaterial-centered infections occur when bacteria attach to an implant or device and form a biofilm by colonizing its surface. Many studies and reviews have addressed this problem of device-related infection (Busscher et al., 1992; Hoyle and Costerton, 1991; Dickinson and Bisno, 1989; Costerton et al., 1987; Gristina, 1987; Dankert et al., 1986; Holmes and Evans, 1986). Examples of implants frequently colonized by biofilm forming bacteria include orthopedic prosthesis and joint replacements (Busscher et al., 1992; Dickinson and Bisno, 1989), pacemaker leads and heart valves (Busscher et al., 1992; Marrie et al., 1982), catheters (Baldassarri et al., 1994; Busscher et al., 1992; Kunin and Steele, 1985; Nickel et al., 1985; Marrie and Costerton, 1984), vascular graphs and endotracheal tubes (Busscher et al., 1992; Dickinson and Bisno, 1989), as well as contact lenses and ocular prosthetics (Busscher et al., 1992; Dickinson and Bisno, 1989; Slusher et al., 1987). Although infections frequently contain more than one microbial species, the most common isolates are members of the skin

flora, *Staphylococcus aureus* (Dickinson and Bisno, 1989; Gristina, 1987; Marrie et al., 1982) and *Staphylococcus epidermidis* (Giridhar et al., 1994; Dickinson and Bisno, 1989; Gristina, 1987; Slusher et al., 1987; Kunin and Steele, 1985; Marrie and Costerton, 1984), or are common environmental microorganisms, like *Pseudomonas aeruginosa* (Costerton and Anwar, 1994; Dickinson and Bisno, 1989; Gristina, 1987; Slusher et al., 1987; Nickel et al., 1985), which ordinarily are pathogenic only in immuno-compromised patients or in those suffering from cystic fibrosis. These device related infections can result in tissue damage, failure of the implant due to lack of tissue integration and inflammatory responses, and life threatening bacteremias. Because the infections are difficult to eliminate with antibiotic treatments, it is often necessary to remove the implanted device until the infection has cleared.

Many techniques have been implemented with hopes of increasing the body's acceptance rates of biomaterials. Chemical modifications of the biomaterial surface (Busscher et al., 1992), seeding the biomaterial with human tissue cells prior to implantation (Busscher et al., 1992), and impregnating the biomaterial with antibacterial agents (Strachan, 1995; Holmes and Evans, 1986) have each met with limited success.

Resistance and Transport

It has been well established that bacterial biofilms are less susceptible to antimicrobials than their freely suspended, planktonic counterparts (Anwar et al., 1989; Nickel et al., 1985). Biofilm bacteria exposed to antimicrobials at concentrations which are typically bactericidal to freely suspended cultures have been shown to survive after treatment for extended periods (Anwar et al., 1992; Nichols et al., 1989; Nichols et al., 1985). This resistance has been attributed to the physiology of the bacteria (Anwar et al., 1992) and limited antimicrobial penetration due to the extracellular polymer matrix or glycocalyx (Hoyle and Costerton, 1991; Evans et al., 1991; Costerton et al., 1984). The susceptibility of bacteria to antibiotics has been shown to be related to their growth rate (Duguid et al., 1992; Evans et al., 1991; Gilbert et al., 1990) and it has been proposed that biofilm cells have slower growth rates which are similar to planktonic cultures in a stationary growth phase (Brown et al., 1988). In addition, younger biofilms have demonstrated less recalcitrance to antibiotics than older biofilms (Anwar et al., 1992; Anwar et al., 1989).

Many studies have tried to elucidate whether the exopolysaccharide matrix of a biofilm provides a transport barrier to antibiotics and whether this limitation is the mechanism of resistance. Yasuda et al. (1994, 1993) established that the rate of antibiotic penetration in a biofilm increased after eradication of the glycocalyx matrix and Costerton et al. (1994) showed that

applying a bioelectric effect enhanced antibiotic efficacy. Costerton et al. (1994) hypothesized that the enhanced efficacy was due increased antibiotic penetration as a result of the application of an electric field. These studies, along with many others, suggest that the exopolysaccharide matrix acts as a transport barrier to the antimicrobial (Hodges and Gordon, 1991; Costerton et al., 1987; Prosser et al., 1987; Slack and Nichols, 1981). However, other studies have stated that the hindered penetration of antibiotics is not significant and that it is not the mechanism of resistance (Nichols, 1994; Nichols et al., 1989; Nichols et al., 1988). Nichols et al. (1989) found that the glycocalyx did not provide any extra resistance to antibiotics when comparing mucoid and non-mucoid *Pseudomonas aeruginosa*. Finally, Dunne et al. (1993) discovered that bactericidal levels of an antibiotic were attained at the biofilm substratum, but that sterilization of the biofilm was not accomplished. These results indicate that molecular diffusion limitations are not the reason for the decreased susceptibility of biofilms to antimicrobial agents.

In a more industrial context, transport studies using a highly reactive antimicrobial, monochloramine, Srinivasan et al. (1995) concluded that transport based explanations of resistance could account for decreased efficacy against thick, robust biofilms, but not thinner ones. DeBeer et al. (1994) found that the limited penetration of chlorine in microbial biofilms was concentration dependent and caused by the neutralization of chlorine in the biofilm matrix. Although most

medical biofilms are in the range of microns to tens of microns (Nichols et al., 1989), a reaction limiting process could be present if the bacteria produced an enzyme, such as β -lactamase, to degrade the antibiotic. Nichols et al. (1988) also suggested that cells could potentially act as sinks by taking up the antibiotic, thereby decreasing the driving force of diffusion. Finally, it has been shown that antibiotics can bind to components of the biofilm resulting in a reduction of the apparent diffusion coefficient, but that the observed delay was not significant and could not account for the reduced efficacy (Nichols et al., 1988).

Whether transport limitation occurs in a biofilm most likely depends upon which organisms are present, the biofilm architecture, and the chemical properties of the antimicrobial.

Modeling Penetration

Mathematical modeling is a useful tool for developing experimental designs, testing hypotheses, and analyzing data. By incorporating multiple simultaneous processes, modeling can simulate experimental systems and allow for manual manipulation of variable parameters. The modeling performed in this study was implemented using AQUASIM. AQUASIM is an aquatic system simulation and data analysis program developed by the Swiss Federal Institute

for Environmental Science and Technology (Reichert, 1994(b)). Simulations, parameter estimations, sensitivity analyses, and uncertainty estimations can be performed using AQUASIM. Previous studies have implemented AQUASIM to study interactions within mixed culture biofilms (Wanner and Reichert, 1995) and to estimate parameters of activated sludge (Reichert et al., 1995).

Other mathematical models have been employed to gain a better understanding of transport processes and antimicrobial resistance within biofilms. Nichols et al. (1989) proposed a model for simulating the effects of binding and uptake upon penetration and the effects of an enzyme catalyzed, irreversible inactivation of the antibiotic. He later implemented this model to predict the penetration time course of an antibiotic (Nichols, 1994). Stewart (1994) modeled antibiotic resistance of *Pseudomonas aeruginosa* biofilms using a dynamic biofilm accumulation model. This model accounted for depletion of the antibiotic by reaction and physiologic resistance of bacteria due to growth rates within the biofilm. Chlorine penetration into artificial biofilms was modeled by Chen and Stewart (1996) using reaction and diffusion theory. In their model, chlorine was neutralized by reacting with the cell mass and the agarose matrix at a reaction rate sufficient to account for the failure of chlorine to penetrate the artificial biofilms during the time course of treatment. Stewart and Raquepas (1995) also implemented reaction-diffusion theory to investigate the potential for transport limitation on the overall rate of biofilm disinfection and the rate of

antimicrobial penetration into the biofilm. They demonstrated that the antimicrobial efficacy decreases sharply as the Thiele modulus exceeds one. In cases where the antimicrobial agent reacted stoichiometrically with the biofilm, it was found that the time scale of penetration could exceed that of transient diffusion by orders of magnitude.

Fluoroquinolone Antibiotics

The antibiotics used in this study, ciprofloxacin and levofloxacin, are classified as quinolones. Entirely synthetic, most quinolones are structurally characterized by the combination of the moiety (A) with a second aromatic or heteroaromatic (B) ring structure (Figure 1). Because ciprofloxacin and levofloxacin bear a fluorine atom at the C-6 position in the ring (Figure 2), they are further classified as fluoroquinolones (Chu and Fernandes, 1989). The presence of this fluorine atom has been shown to increase potency (Reese and Maxwell, 1991).

Quinolone antibacterial agents exhibit bactericidal activity against a broad spectrum of Gram-positive and Gram-negative bacteria. Bacterial DNA gyrase (topoisomerase II) is a primary target for the quinolone agents and is central to its mechanism of action. Topoisomerase is an enzyme which alters the number

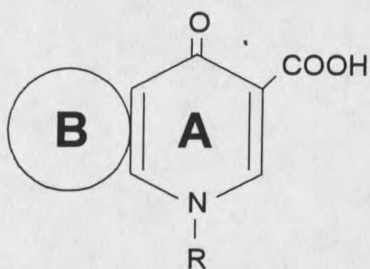
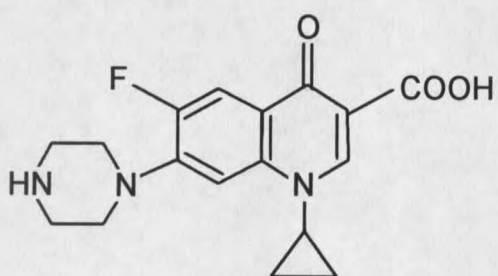


Figure 1: Generic chemical structure for quinolone antibiotics.

Ciprofloxacin



Levofloxacin

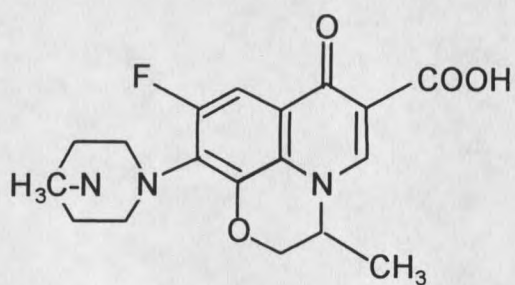


Figure 2: Chemical structures of the fluoroquinolones, ciprofloxacin and levofloxacin.

of times a single strand of a DNA duplex winds around its complementary strand. The level of DNA coiling is important in regulating the replication of bacterial DNA and the transcription of certain genes. Exposure of bacteria to quinolone agents causes DNA damage by affecting its coiling and generally results in rapid cell death (Wolfson and Hooper, 1989). It has been proposed that hydrogen bonding is the mode of interaction between the DNA and the fluoroquinolones (Shen et al., 1989).

DNA gyrase is essential for DNA replication, so antibiotics that inhibit the DNA gyrase also inhibit replication (Reese and Maxwell, 1991). Although this mechanism of killing requires the replication and new synthesis of bacterial protein(s) (Wolfson and Hooper, 1989), there has been evidence that modern quinolones, such as levofloxacin and ciprofloxacin, are able to kill bacteria which are not dividing or actively synthesizing protein or RNA (Morissey and Smith, 1994; Lewin and Amyes, 1990). There is also evidence that quinolone agents differ in their abilities to kill the same bacterial strain. Maximal killing tends to occur at approximately 30- to 60-fold above the minimal inhibitory concentration (MIC) while treatment with even higher concentrations can result in a paradoxical killing effect. In the paradoxical killing effect, treatment with excessive concentrations of antibiotic can be counterproductive. Bacteria exposed to an antibiotic at concentrations exceeding 100 times the MIC may be less susceptible to the antimicrobial agent than bacteria treated with

concentrations closer to the MIC (Holm et al., 1991). This response is thought to occur due to an inhibition of RNA and protein synthesis by high quinolone concentrations (Crumplin and Smith, 1975).

Mechanisms of bacterial resistance to quinolones include alterations to the DNA gyrase and the permeability of the bacterial outer membrane. One chromosomal resistance mutation resides in the structural gene for DNA gyrase A protein, *gyrA* (Reese and Maxwell, 1991). The second class of resistance, decreased drug permeation, has been associated with an outer membrane porin protein on *E. coli*, *OmpF* (Nikaido, 1989). Thus far, there has been no evidence of quinolone drug destruction (i.e. by an enzyme) as a mechanism of bacterial resistance (Wolfson and Hooper, 1989). Although quinolones can kill a broad spectrum of bacterial cells, they remain relatively non-toxic to human cells because the eukaryotic topoisomerase enzyme has a different sensitivity to the drug (Gootz et al., 1990).

ATR-FTIR Technique of Monitoring Antimicrobial Penetration

Most studies of antimicrobial penetration have been performed on micro-colonies of cells (Nichols et al., 1989; Nichols et al., 1988) and on biofilms or micro-colonies grown on either permeable membrane filters (Yasuda et al., 1994; Yasuda et al., 1993; Nichols et al., 1989) or dialysis membranes (Dunne et

al., 1993; Nichols et al., 1988). Artificial biofilms (cells suspended in a gel) have also been used (Chen and Stewart, 1996). The ATR-FTIR technique proposed by Suci et al. (1994) is advantageous in that antibiotic penetration of a biofilm grown on a solid inert substratum can be monitored non-invasively. Such a system allows the study of a more "realistic" biofilm where the biofilm's architecture has been dictated by the supply of nutrients and antimicrobials from only one side of a solid substratum.

Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) has previously been used to characterize thin polymer films (Baty et al., 1996; Suci and Geesey, 1995) and to study protein interactions and conformational changes (Ong et al., 1994). Protein adsorption has been monitored over time using ATR-FTIR methodologies (Baty et al., 1996; Burmeister et al., 1996). The use of ATR-FTIR to monitor the adhesion of microbial cells and subsequent biofilm formation was first employed by Nivens et al. (1993). Suci et al. (1994) took this concept one step further to monitor the penetration of an antibiotic through an established biofilm.

A more detailed explanation of the ATR-FTIR technique is provided in the next chapter of this thesis, ATR-FTIR Theory.

THEORY

ATR-FTIR Theory

Attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy utilizes the frequency information of absorbed infrared radiation to determine the molecular structure of organic compounds.

The infrared (IR) portion of an electro-magnetic spectrum, lying between visible and microwave regions, contains wavenumbers ranging from 12,000 to 10 cm^{-1} ($0.78\text{-}1,000\text{ }\mu\text{m}$). The majority of IR spectroscopy analytical applications utilize the region from $4,000\text{ to }670\text{ cm}^{-1}$ ($2.5\text{-}15\text{ }\mu\text{m}$) (Skoog, 1985; Silverstein et al., 1981). In IR spectroscopy, radiation is absorbed by quantum energy levels of organic molecules which are associated with atomic vibrations (middle range IR $4,000\text{-}200\text{ cm}^{-1}$ ($2.5\text{-}50\text{ }\mu\text{m}$)) and rotations (far IR $200\text{-}10\text{ cm}^{-1}$ ($50\text{-}1,000\text{ }\mu\text{m}$)) (Skoog, 1985). Vibrational motion is due to the oscillations of atoms in a molecule and rotational motion is due to the rotation of the molecule about its center of mass. Every atomic vibrational level has a set of closely spaced rotational energy levels. The energies of the rotational levels are much lower than those of the vibrational levels. In order to absorb IR radiation, a molecule must undergo a net change in dipole moment as a consequence of its vibrational

or rotational modes. When the frequency of the IR radiation matches the natural vibrational frequency of a molecule, the molecule may become vibrationally excited resulting in an absorption of IR radiation. Vibrational excitation occurs when a transition is made from a particular rotational energy level in a specific vibrational state to a different rotational level in an excited vibrational state. Under these conditions, a net transfer of energy occurs that results in a change in the amplitude of the molecular vibration. Only molecules which have dipole moments are active in the infrared region and produce an absorbance spectrum. The frequency or wavelength of IR absorption depends upon the relative masses, the geometry, and the bond force constants of the atoms.

An intermediate portion of the middle IR spectrum is often used for compound identification. This range from $1500\text{--}700\text{ cm}^{-1}$ (6.7 to $14.3\text{ }\mu\text{m}$), generally referred to as the fingerprint region, contains complex absorption patterns which are unique for most organic molecules (Skoog, 1985; Silverstein et al., 1981)

A Fourier transform (FT) is a mathematical tool that relates a continuous function in one domain (time) to its components in another domain (frequency). FTs are used to convert the detected time-dependent composite signal to an IR spectrum based upon its frequencies. FT techniques in IR spectroscopy are highly efficient because they allow the spectrometer to operate in the time domain where measurements of all frequencies are collected simultaneously

instead of one frequency at a time. Consequently, this technique provides faster data acquisition, as well as greater sensitivity and resolution.

Attenuated total reflection (ATR) occurs when a highly directed IR beam undergoes total internal reflection within a material of high refractive index (germanium or zinc selenide) which is surrounded by a medium of lower refractive index (water or air). The high refractive index material is often a prism or internal reflection element (IRE). By adjusting the geometry of an external mirror assembly, the IR beam can be directed with its angles of incidence greater than the critical angle of reflectance. When this condition is met, all the energy of the IR beam is reflected within the IRE. Multiple reflections occur within the IRE and an evanescent field forms at the IRE surface. If a molecule in a sample lies within the extent of the evanescent field (approximately $1\text{ }\mu\text{m}$ from the IRE surface) some of the IR beam can be absorbed by the molecule. The detected IR beam provides an absorption spectrum that is indicative of the sample's molecular structure. ATR-FTIR is a real-time, in-situ, non-destructive measurement technique which requires no extrinsic label to be added to the sample for detection. The limited sampling depth sensitivity, equivalent to a fraction of a wavelength (approximately $1\text{ }\mu\text{m}$), makes ATR-FTIR an ideal tool for monitoring the appearance of a molecule at the base of a thin film.

A diagram of FTIR instrumentation is shown in Figure 3. IR radiation is produced by an electrically heated source. Mirrors divide the radiation from the

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy

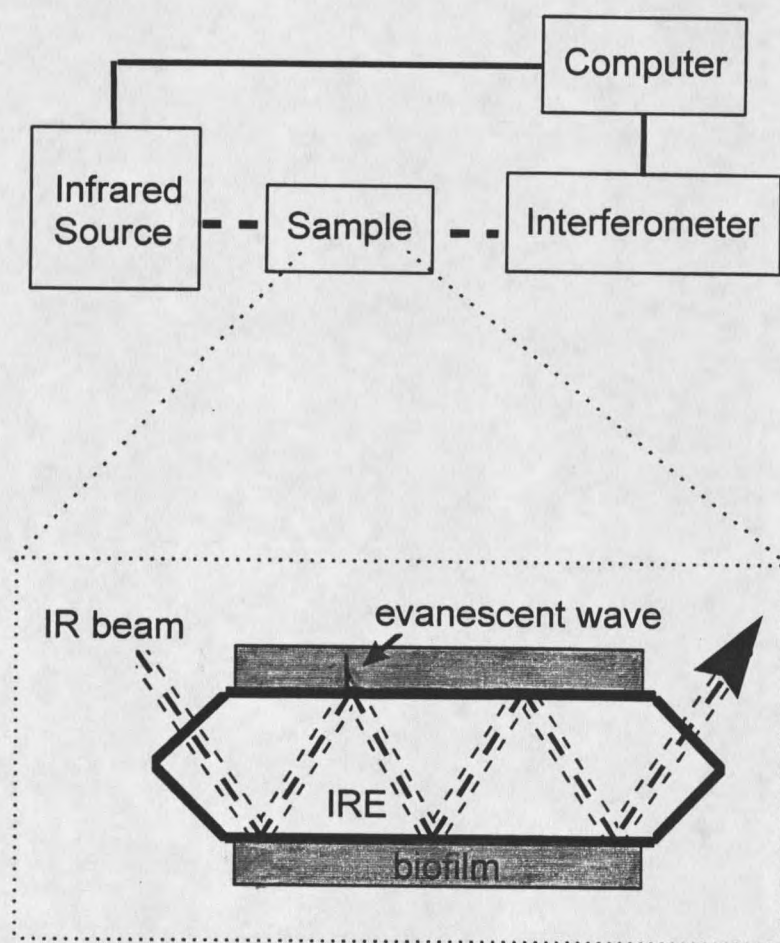


Figure 3: Schematic diagram of ATR-FTIR spectroscopy.

source into beams and focus these beams into the sample area. Here the beam interacts with the sample. The beam is then collected as a transmittance spectrum using interferometry (Strobel and Heineman, 1989). A computer calculates the fast FT of the signal and later can convert the transmittance spectrum to one of IR absorbance.

Modeling Theory

The computer model, AQUASIM™ (Reichert, 1994(a)), was tailored to match experimental conditions and used to explore antibiotic transport processes in a biofilm. The model simulated a biofilm reactor compartment which represented a well mixed reactor with biofilm growing on the walls (Reichert, 1994(b)). This compartment was sub-divided into two zones, bulk liquid and biofilm, which were linked by coupled mass transport equations. The bulk liquid zone simulated a well-mixed, constant volume, stirred tank reactor (or chemostat) subject to continuous flow conditions. The biofilm zone was simulated by a planar sheet of uniform thickness containing cells and particulates. A liquid boundary layer separated the biofilm and bulk liquid zones. The boundary layer was based on diffusive mass transfer resistance and fulfilled the condition that the mass flux through the boundary layer equal the interfacial transfer rate of the biofilm (Wanner and Reichert, 1995).

For this study, antibiotic was added to the bulk fluid by the following step dosing procedure:

$$\begin{aligned} A_{in}(t) &= 0 & \text{for } t < 0 \text{ min} \\ A_{in}(t) &= A & \text{for } 0 < t < 30 \text{ min} \\ A_{in}(t) &= 0 & \text{for } t > 30 \text{ min} \end{aligned}$$

where A_{in} represents a known antibiotic dosing concentration. There were no cells or particulates in the bulk fluid to interact with the antibiotic or interfere with its transport to the biofilm zone.

Interactions within the biofilm compartment were represented by rate processes (Reichert, 1994(b)). Dynamic processes were used to describe growth and transport phenomena because of their dependence upon time. The basic model contained the processes of bulk flow in and out of the reactor, cell growth and substrate consumption by cells following Monod kinetics, and Fickian diffusion of the antibiotic through the biofilm.

Antibiotic transport by Fickian diffusion was modeled by the expression:

$$\frac{\partial A}{\partial t} = D_A \frac{\partial^2 A}{\partial z^2} \quad (1)$$

where A was the concentration of free antibiotic, D_A was the diffusion coefficient of the antibiotic in water, and t and z were time and space constants, respectively. Within the biofilm, the diffusion coefficient was multiplied by a ratio which related the permeability of the biofilm to that of the bulk fluid. To this basic model, other transport processes were added including adsorption and

desorption to simulate the binding of antibiotics to biomass. These processes were activated or inactivated by choice in the model simulations.

The first order processes of adsorption and desorption were described by the following set of equations:

$$\frac{\partial A}{\partial t} = D_A \frac{\partial^2 A}{\partial z^2} - K_S * A * S + K_D * A_S \quad (2)$$

$$\frac{\partial A_S}{\partial t} = K_S * A * S - K_D * A_S \quad (3)$$

$$\frac{\partial S}{\partial t} = K_D * A_S * Y_{SA} - K_S * A * S * Y_{SA} \quad (4)$$

where A was the concentration of free antibiotic, A_S was the concentration of bound antibiotic, and S was the concentration of the binding particulate. K_S and K_D were rate constants for the sorption and desorption processes, respectively.

The yield, or ratio, of grams sorbing particulates per gram antibiotic was expressed by Y_{SA} and set equal to one, for simplicity, during all simulations. The total amount of antibiotic in the reactor was equivalent to the sum of sorbed antibiotic and free antibiotic.

Boundary conditions for the model included a no flux condition at the biofilm-substratum interface,

$$\left. \frac{\partial A}{\partial z} \right|_{z=0} = 0 \quad \text{for } t > 0, \quad (5)$$

and a matching flux condition at the biofilm-bulk fluid interface,

$$R \frac{\partial A}{\partial z} \Big|_{z=L_f} = \frac{A_{bulk} - A|_{z=L_f}}{L_l} \quad \text{for } t > 0. \quad (6)$$

L_f and L_l represented the thickness of the biofilm and liquid layer, respectively.

R was the biofilm-bulk fluid effective diffusivity ratio.

METHODS

Microbial Species

The *Pseudomonas aeruginosa* strain used for this study (ERC-1) was obtained from the National Science Foundation Engineering Research Center for Biofilm Engineering, Montana State University, Bozeman, MT. Cultures of *P. aeruginosa* were stored as frozen stocks maintained in a solution of 2% peptone and 20% glycerol at -70°C.

Solutions

Medium

A minimal salts medium with glucose as the sole carbon source was used for culturing planktonic and biofilm bacteria. The nutrient medium, excluding the glucose, CaCl_2 , and Wolfe's mineral salt ingredients, was prepared in 10 and 20 L Nalgene carboys and autoclaved at 121°C for 4.5 h. A concentrated solution of glucose and CaCl_2 , along with the Wolfe's mineral salts solution, was filter sterilized through a 0.2 μm sterile, syringe filter (Acrodisc, Gelman Sciences) and added to the sterile medium by injection through a septum. The nutrient

medium was modified from that described by Suci et al. (1994); the amount of potassium phosphate was reduced to prevent precipitation of the antibiotics. The composition of the nutrient medium per liter water was as follows: 0.28 g D-glucose, 0.017 g CaCl_2 , 0.24 g NH_4Cl , 0.051 g $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 0.1 g K_2HPO_4 (anhydrous), and 0.6 ml Wolfe's mineral salts solution. The final medium pH was between 7.1 and 7.2. The composition of Wolfe's mineral salts solution per liter water was as follows: 1.5 g nitriloacetic acid, 0.5 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 1.0 g NaCl , 0.1 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1 g CaCl_2 , 0.1 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.01 g $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$, 0.01 g H_3BO_3 , and 0.01 g $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$. Water was ultra pure Milli-Q™ water: de-ionized, carbon filtered, cell filtered, and subjected to reverse osmosis.

Phosphate Buffer

The phosphate buffer solution used in all dilution series contained 0.1 g K_2HPO_4 (anhydrous) per liter Milli-Q water. This was the same concentration used in the medium. The buffer solution was pH adjusted to 7.1-7.2, distributed into dilution tubes, and autoclaved for 25 minutes at 121°C.

Antibiotic Solutions

Ciprofloxacin powder was obtained from Bayer (Leverkusen, Germany), ciprofloxacin liquid 10 mg ml^{-1} stock solutions were obtained from Miles Canada

(Etobicoke, Canada), and levofloxacin powder was supplied by the R.W. Johnson Pharmaceutical Research Institute (Spring House, PA). The powdered form of ciprofloxacin was used for all transport experiments and the liquid stock was used in the planktonic efficacy studies. Care was taken to achieve the same molar concentration for each antibiotic. The calculated molecular weights of ciprofloxacin and levofloxacin were 331.14 and 361.16 g mole⁻¹, respectively. The molar concentration of 1.5×10^{-3} moles l⁻¹ corresponds to 496.7 µg ciprofloxacin ml⁻¹ and 541.7 µg levofloxacin ml⁻¹ (approximately 500 µg ml⁻¹), 7.5×10^{-4} moles l⁻¹ to 245.0 µg ciprofloxacin ml⁻¹ and 267.3 µg levofloxacin ml⁻¹ (approximately 250 µg ml⁻¹), and 3.0×10^{-4} moles l⁻¹ to 99.3 µg ciprofloxacin ml⁻¹ and 108.4 µg levofloxacin ml⁻¹ (approximately 100 µg ml⁻¹). For simplicity in the following manuscript, the concentrations of levofloxacin and ciprofloxacin will be referred to by their approximate mass concentrations, 100, 250, and 500 µg ml⁻¹.

Cleaning Procedures

Internal Reflection Element

Internal reflection elements (IREs) were cleaned prior to each experiment by a slight variation of the method for cleaning germanium presented in Baty et al. (1996). First, the IREs were submerged in a base-bath cleaning solution of isopropyl alcohol saturated with potassium hydroxide and sonicated for 10

minutes. Germanium IREs were placed vertically in teflon holders during sonication regimes. The IREs were then dip rinsed in a beaker of Milli-Q cell free water, placed in a fresh Milli-Q water bath and sonicated for 10 minutes. Next, IREs were gently scrubbed with a concentrated cleaning solution (Micro, International Products Corporation, Burlington, NJ) using cotton-tipped applicators (Puritan, Fisher Scientific), rinsed in a Milli-Q water jet stream from a water bottle, and exposed to a sonication series of three Milli-Q water baths for 10 minutes each. Finally, the IREs were sonicated for 10 minutes in a 95% ethanol bath. Once clean, the crystals were stored under a glass beaker until needed.

Circle-cell Assembly

The circle-cell assembly was cleaned by submersion and sonication (10 min) in a base-bath cleaning solution of isopropyl alcohol and potassium hydroxide. The circle cell was then rinsed under a steady stream of warm tap water for 3 minutes, placed in a 95% ethanol bath, and sonicated (10 min). Once clean, the circle-cell was allowed to air dry.

Tubing and Flasks in Flow System

All tubing and flasks were cleaned immediately following the experiment by pumping a solution of 5% nitric acid through the system for 30 to 45 minutes

at 3.2 ml min^{-1} . This was followed by a 1 to 1.5 hour rinse with Milli-Q water at the same flow rate.

Sterilization of Flow System, Circle-cell, and IRE

The flow system including the chemostat, antibiotic reservoirs, and tubing were autoclaved for 30 minutes at 121°C after cleaning. The circle cell assembly containing the IRE was sterilized by filling the chamber with 70% ethanol and soaking for 3 hours immediately before introduction of the medium and bacterial inoculum.

Experimental Designs

Antibiotic Efficacy Against Planktonic Organisms

Planktonic efficacy experiments were designed to mimic the biofilm experiments. Care was taken to keep the ratio of the antibiotic mass that a cell was exposed to during a 30 minute biofilm treatment to the total number of cells in the biofilm the same in the planktonic experiments. Ten ml samples containing approximately $5 \times 10^7 \text{ cells ml}^{-1}$ were drawn from a batch culture near the end of exponential growth phase and exposed to antibiotic concentrations of 100, 250, or $500 \mu\text{g ml}^{-1}$ for 30 minutes. Samples were then spun down for 10 minutes in a Sorvall Instruments RC-3B refrigerated centrifuge at room

temperature and resuspended in fresh medium. This process was repeated for a total of 4 rinses to simulate the one hour rinse in the biofilm experiments. After the fourth rinse, a dilution series was made to assess viable and total cell counts.

Flow System for Transport Experiments

Schematic diagrams of the experimental set-ups for colonized and uncolonized IRE experiments are shown in Figures 4 and 5. The sample chamber containing the circle cell and the IRE had a liquid volume of 0.289 ml. Under flow conditions of 3.2 ml min^{-1} , the sample chamber had a residence time of 5.4 sec, a sheer rate of 41 sec^{-1} , and a Reynolds number of 9 indicating laminar flow. All flow rates were controlled by Masterflex peristaltic pumps (Cole-Parmer Instruments). Medium was fed to the 500 ml continuous culture (chemostat) at a rate of 0.83 ml min^{-1} (chemostat dilution rate, 0.10 hr^{-1}). The inoculum flow rate leaving the continuous culture and branching to each bacterial feed was 1 ml min^{-1} (0.5 ml min^{-1} to each branch). This inoculum was supplemented with medium and the flow rate of the inoculum-medium mixture through each sample chamber was 3.2 ml min^{-1} . Once the inoculation ceased, fresh medium was supplied to each sample chamber at 3.2 ml min^{-1} . Finally, antibiotics were delivered and washed out with medium at rates of 3.2 ml min^{-1} in each sample chamber.

For each experiment, the culture medium was transferred aseptically from the main reservoir to the antibiotic reservoir to ensure that the medium containing the antibiotic was identical (except for the presence of the antibiotic) to the culture medium. The appropriate amount of each antibiotic was added to the respective reservoir 30 minutes prior to delivery to the sample chamber. The medium containing antibiotic was delivered to the sample chambers by removing and relocating a clamp to block the non-antibiotic medium feed. To provide the rinse, the clamp was switched back to block the antibiotic medium feed. Care was taken to maintain uninterrupted flow during such transitions. Flow rates were monitored periodically by counting effluent drops over time and by counting drops in glass break tubes placed in the tubing lines.

Antibiotic Transport To Colonized IREs

A chemostat containing 500 ml of sterile medium was inoculated with 1 ml of a *Pseudomonas aeruginosa* suspension (from frozen stock) and grown in a batch culture until turbid. Twenty-four hours after batch culture inoculation, continuous flow to the chemostat was turned on. Sterile medium was sparged with filtered air (2 l min^{-1}) and fed to the chemostat at a dilution rate of 0.1 hr^{-1} . The chemostat was well mixed with a magnetic stir bar and sparged with filtered air (1 l min^{-1}). All air was filtered with Whatman HEPA-Vent™ air filters (0.3 μm particle retention, Fisher Scientific). After the chemostat had maintained steady-

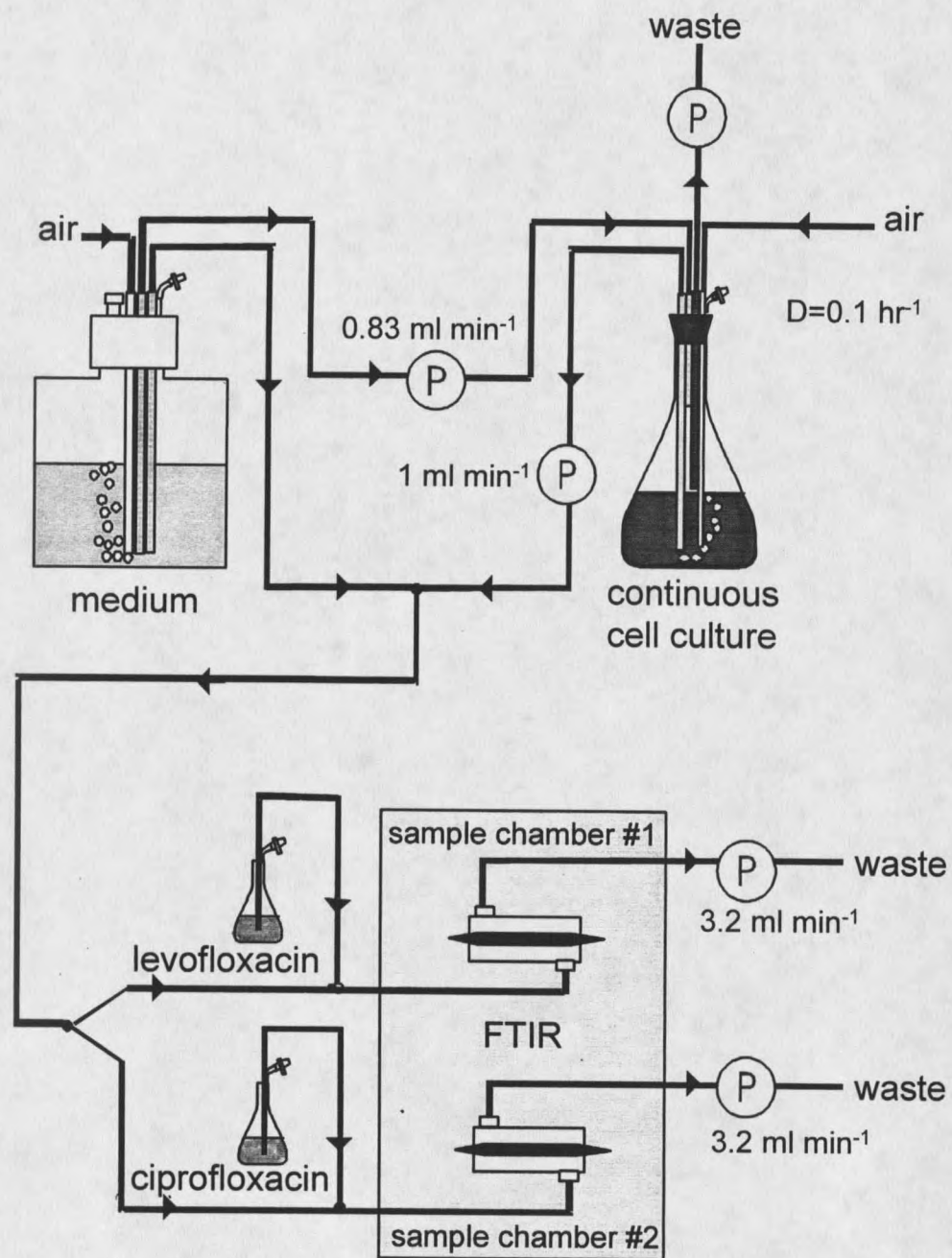


Figure 4: Schematic diagram of colonized IRE experimental system. The symbol (P) denotes a pump.

state operation for 3 residence times (30 h), inoculation of the two FTIR sample chambers began. The cell inoculum from the chemostat was supplemented with fresh, sterile medium and was supplied to each FTIR sample chamber for 20 hours. FTIR spectra were acquired each hour beginning with the start of the flow cell inoculation. After 20 hours, the cell feed from the chemostat was turned off and fresh medium continued to be supplied to each flow cell for an additional 30 hours. During this time, biofilm development was monitored by sampling FTIR spectra each hour. Once the biofilm was 50 hours old, the antibiotics were introduced into their respective sample chambers at a rate of 3.2 ml min^{-1} . The antibiotics were dosed for 30 minutes and were followed by a 60 minute rinse of sterile medium. FTIR spectra were collected every 36.6 seconds during the dosing and rinse periods. At the end of the rinse period, the flow system was turned off and the biofilms were assayed for viability. The same procedure was followed for the control experiments, except that there was no antibiotic in the antibiotic reservoir, only sterile medium. Figure 6 is a diagram illustrating the experimental method of biofilm development and antibiotic delivery within the FTIR sample chamber.

Antibiotic Transport To Uncolonized IREs

Uncolonized IRE experiments were performed to monitor the delivery of the antibiotic to the substratum when a biofilm was not present. The flow

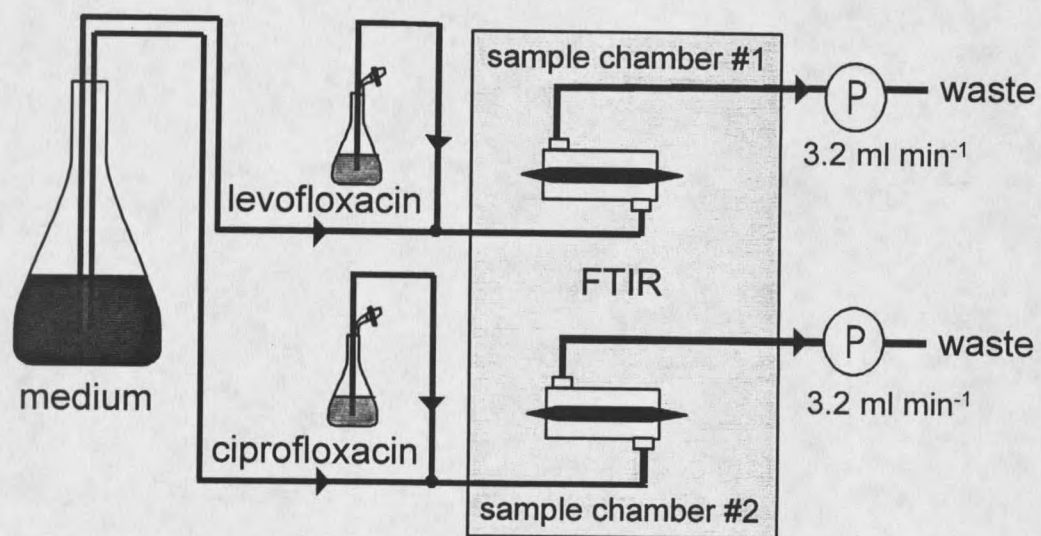


Figure 5: Schematic diagram of uncolonized IRE experimental system. The symbol $\textcircled{\text{P}}$ denotes a pump.

system, as shown in Figure 5 was slightly simpler than that of the biofilm experiments because a cell inoculum was not needed. The types of tubing, lengths, and diameters were kept the same as in the biofilm experiments, as was the flow rate of 3.2 ml min^{-1} . The flow system, circle-cell, and IRE were sterilized before the experiment according to the previously stated method. Medium was pumped through the sample chamber for 3 minutes and a background FTIR spectra was taken. The antibiotics were then dosed for 30 minutes followed by a 60 minute rinse of sterile medium. FTIR spectra were collected every 36.6 seconds during the dosing and washout periods.

Cell Enumeration

Scraping of Biofilm

The biofilm was scraped from the germanium FTIR IRE or coupon with a sterile, disposable cell scraper (Fisherbrand, Fisher Scientific) into 5 ml of phosphate buffer solution. The IRE or coupon was then rinsed a second time with 5 ml of buffer solution to collect any cells that may have escaped the first scraping. Next, cell suspensions were sonicated for 5 minutes to break up the clusters of cells.

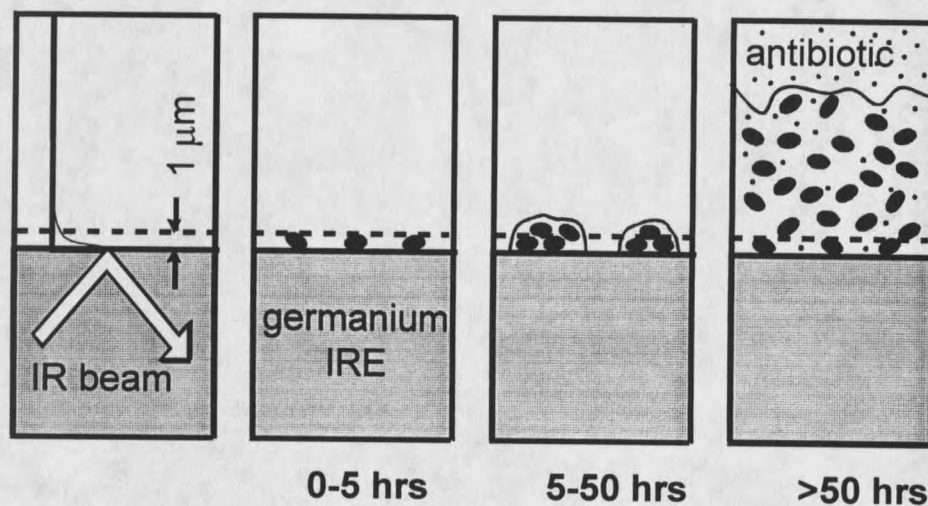


Figure 6: A diagram illustrating the colonized IRE experimental method. Upon inoculation, bacteria attached to the germanium IRE (0-5 hrs) and grew to form colonies (5-50 hrs) which covered more of the substratum. Colonization within 1 μm of the substratum was monitored using ATR-FTIR spectroscopy. Once a mature biofilm had developed (>50 hrs), completely covering the substratum, the antibiotic was administered and its penetration monitored using ATR-FTIR spectroscopy.

Viable Cells

A standard dilution series was made from the sonicated cell suspension sample. Relevant dilutions were then plated onto Difcobacter R2A™ agar plates by the drop plate method, in which 20 µl samples from a particular dilution are pipetted and dropped onto a plate. This procedure was performed in triplicate. Plates were incubated for 3 days at room temperature. Drops that contained between 3 and 30 colonies were enumerated and using the appropriate dilution factor, converted to obtain counts in cfu ml⁻¹.

Total Cells

Certain dilutions from the viable cell dilution series were saved for total cell enumeration and fixed with a 2% glutaraldehyde solution. A black polycarbonate membrane (pore size 0.22 µm and 25 mm diameter, Poretics Corporation) was placed on a cell free glass Millipore filter apparatus and a filtration column was attached. 0.5 ml of the fixed cell suspension was placed in the membrane filtration column, followed by 0.5 ml of 0.05% acridine orange (Sigma, St. Louis, MO) solution. The combination was allowed to stand for 7 minutes prior to filtration. Once the solution was removed by vacuum filtration, the membrane was mounted to a slide in the following manner: a drop of oil (R.P. Cargille Laboratories; Inc., non-drying immersion oil type FF) was placed on a microscope slide, the filter membrane was placed on top of this drop, and a

second drop of oil was placed on top of the membrane and covered with a glass cover slip. The slide was then examined under fluorescent microscopy.

Bacteria in twenty grids (60 μm x 60 μm) per slide were counted using an Olympus model BH-2 microscope with an Olympus model BH2-RFL-T2 mercury lamp, and an Olympus 100X D Plan APO 1.30 NA oil immersion objective magnified 1.5 times (all equipment from Olympus Optical Company, Ltd., Japan). Total counts (# cells/grid) were then converted to total cells ml^{-1} using following equation:

$$\frac{\text{total cells}}{\text{ml}} = \frac{(\# \text{ cells/grid}) * (\text{conversion factor}) * (\text{dilution})}{\text{sample volume}} \quad (7)$$

where the conversion factor was 6.305×10^4 and the sample volume was 0.5 ml.

Examination of Biofilm Architecture

To help characterize the biofilm that forms on the FTIR germanium IRE, the architecture of thin sections of intact biofilm was examined by sampling germanium coupons placed in the effluent tubing approximately 12 inches behind the sample chamber. Two germanium coupons were placed in each effluent. The first coupon (closest to the sample chamber) was used to obtain total and viable cell numbers. The same viability assay was performed on this coupon as described previously for the FTIR IRE. The second coupon (furthest from the sample chamber) was used for biofilm morphology analysis by either

confocal microscopy examination or by the cryoembedding-cryosectioning technique.

Confocal Microscopy

Following the completion of a colonized IRE-FTIR transport experiment, the second coupon was removed from each effluent tube, placed in a phosphate buffer bath, and fixed with 5% glutaraldehyde. The biofilm coupons were kept hydrated until examination with a Biorad MRC 600 confocal laser scanning microscope (Biorad, Hercules, CA).

In preparation for viewing with the confocal microscope, the coupons were rinsed three times with a trizma base solution (0.12% g trizma base, 0.9% NaCl, pH=7.4) to remove the glutaraldehyde solution. The coupons were then covered with a propidium iodide stain solution (1 ml TBS, 10 μ l PI stock) for 15 minutes. After pipetting off the propidium iodide solution, the coupons were rinsed once with TBS and mounted to a glass microscope slide with double-sided tape for inverted viewing.

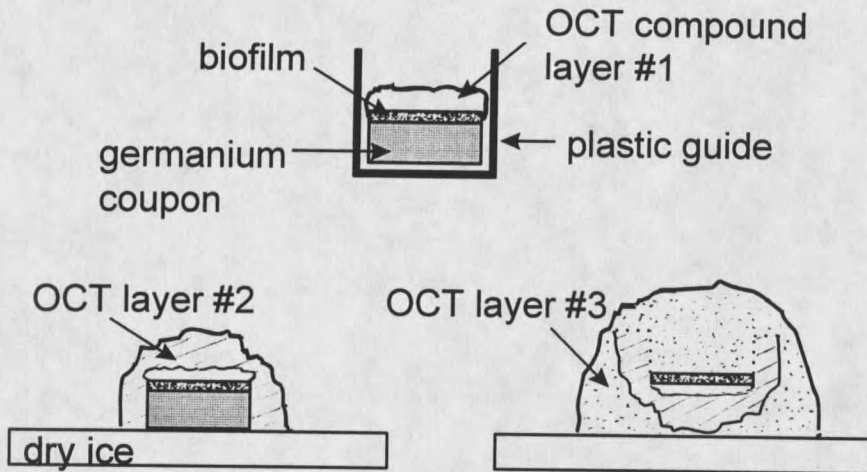
The confocal laser scanning microscope was used to "optically section" the structure of the biofilms. Images were taken at various planes within the biofilm providing a plan or "top" view. The substratum of the coupon was examined to determine whether a confluent layer of cells occupied the surface, or whether there were any uncolonized areas on the germanium substratum.

Also, z-sections were obtained to provide images of the biofilm cross-section. The z-sections allowed us to estimate the thickness of the biofilm and gain information on its structural heterogeneity. Direct images and Kalman averages were taken and, depending upon the image quality, sometimes images were enhanced by performing a contrast-stretch procedure.

Cryoembedding-cryosectioning

In the cryoembedding-cryosectioning technique, the second germanium coupon from each effluent tube was placed biofilm side up in a plastic guide, coated with a thin layer of clear OCT embedding agent (Tissue-Tek OCT compound, Miles Inc., IL), and allowed to sit for approximately 3 minutes. This coupon was then gently placed on a slab of dry ice, biofilm side up, and again coated with OCT. Once the embedding agent had turned opaque white indicating that it was frozen (5-10 min), the coupon was pried away from the frozen embedding agent with the aid of tweezers. The frozen OCT shell was then placed, substratum side up and coated with OCT. Once frozen, this side was marked with an indelible marker to indicate the substratum side of the specimen. The embedded biofilms were stored at -70°C until sectioning. Figure 7 illustrates the cryoembedding and cryosectioning procedures. Cross-sections of the biofilm, 5 μm thick, were made with a cryostat (Reichert-Jung Cryocut 1800, Leica), placed on a polylysine coated microscope slide (Superfrost Plus, Fisher Scientific), and

CRYOEMBED



CRYOSECTION

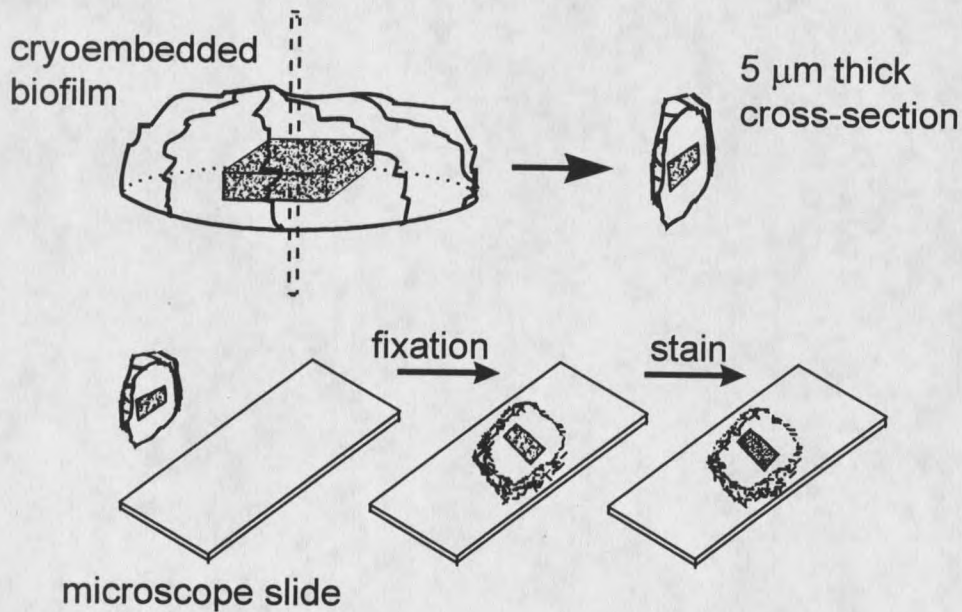


Figure 7: Illustration of the cryoembedding and cryosectioning procedures.

fixed for 10 minutes at 4°C in a solution of 10% formaldehyde, 5% glacial acetic acid, and 85% ethanol (Wentland, 1995). The slides were then rinsed twice with a solution of 85% ethanol and allowed to air dry. Staining the section before microscopic examination involved coating each section with 20 μ l of a 0.0004% acridine orange solution and letting it stand for 2 minutes. Excess stain was removed by carefully blotting each section with Kimwipe tissue paper (Kimberly Clark, Fisher Scientific). Dry sections were then covered with a glass cover slip and examined under fluorescent microscopy.

FTIR Spectroscopy

A Perkin-Elmer model 1800 FTIR spectrophotometer equipped with a liquid-N₂-cooled, medium range mercury-cadmium-telluride detector (5,000 to 580 cm^{-1}) was used to collect the FTIR spectra. This spectrophotometer model is a double-beam instrument which allows for a direct, simultaneous comparison of two sample chambers which have been exposed to identical experimental conditions. Each sample chamber was a Micro-Circle cell designed to enclose a cylindrical germanium internal reflection element (IRE) (Spectra Tech, Stamford, CT).

Acquisition of FTIR Spectra

All FTIR spectra during IRE colonization and antibiotic transport monitoring were collected digitally on a personal computer.

Bacterial accumulation on the IRE was monitored by acquiring FTIR spectra for each sample chamber every hour, beginning with the bacterial inoculation. Spectra were acquired at a resolution of 2 cm^{-1} and an interval of 2 cm^{-1} over a range of $4,000$ to 700 cm^{-1} . Fifty interferograms were collected and averaged per spectrum. Immediately prior to the introduction of bacteria into the sample chamber, a background spectra of sterile medium was taken under the same flow conditions and FTIR parameters to serve as a reference.

Antibiotic transport to the IRE surface and the subsequent rinse were monitored by acquiring FTIR spectra for each sample chamber at a resolution of 8 cm^{-1} and interval of 4 cm^{-1} every 36.6 seconds. One interferogram was acquired per spectrum at a range of $2,500$ to 800 cm^{-1} . Immediately prior to the introduction of the antibiotics into the sample chambers, background spectra of the 50 hour old biofilm and sterile media were collected for each sample chamber.

Analysis of FTIR Spectra

All bacterial growth and antibiotic transport spectra were referenced to their respective background spectra and differences were taken via in-house

custom software. Water vapor bands were removed by subtraction of a pure water vapor spectrum. Residual water adsorption was removed by subtracting a pure water spectrum which was ratioed according to the areas of a peak centered at $2,120\text{ cm}^{-1}$. Difference spectra, which had been corrected for water vapor and residual water, were used to obtain peak areas. Areas of spectral peaks were computed using software by connecting the two limit points of integration with a linear baseline. Figure 8 illustrates this procedure. The amide II band, indicative of bacterial protein, was used to monitor the bacterial colonization of the IRE. Its limit points were $1,580$ to $1,500\text{ cm}^{-1}$. To monitor the antibiotic transport, limit points of $1,350$ to $1,325\text{ cm}^{-1}$ were used for ciprofloxacin and 995 to 965 cm^{-1} for levofloxacin. These antibiotic bands were chosen because they appeared in regions which were relatively free from bacterial interference. All band area values were normalized to the water absorption band ($1,740$ to $1,540\text{ cm}^{-1}$) area obtained from the first background spectra of sterile medium at the beginning of each experiment. The normalizing equation was as follows: Normalized band area = band area * $(300/\text{water band area})$. The value of 300 is an arbitrary, normalizing factor. The normalized band areas were then plotted against time to obtain lateral growth curves and antibiotic transport curves.

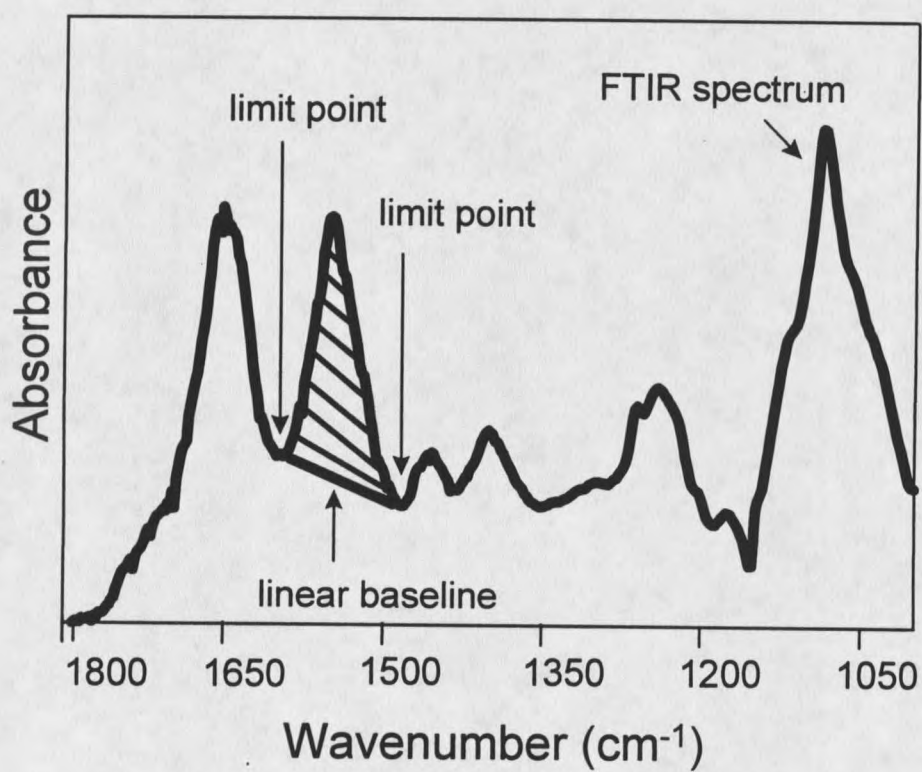


Figure 8: Illustration of the technique for computing spectral band areas.

Modeling

Interpretation of experimental data using AQUASIM

Experimental antibiotic transport data were fit to several mathematical models using the AQUASIM biofilm modeling software (Wanner and Reichert, 1996). Specific variables in the mathematical models could be manipulated manually or by parameter estimations to achieve a fit. Parameter estimations were performed on antibiotic transport curves for the active transport processes of (1) diffusion and (2) diffusion, adsorption, and desorption. Parameters allowed to vary in these fits were the adsorption and desorption rate constants, the concentration of binding sites, and the antibiotic diffusion coefficient.

Experimental data was imported into AQUASIM as a real list variable which could then be used as target values during parameter estimation (Reichert et al., 1995). Parameters, termed constant variables in AQUASIM, were manipulated during parameter estimations to fit the experimental data with the weighted least squares method. In this method, the sum of the squares of the weighted deviations between experimental measurements and simulated calculations was minimized according to the following equation:

$$X^2(p) = \sum_{i=1}^n \left(\frac{f_{meas,i} - f_i(p)}{\sigma_{meas,i}} \right)^2 \quad (8)$$

where $f_i(p)$ was any variable at a given time and location in the compartment as a function of the parameters $p=(p_1, \dots, p_m)$. The values, $f_{meas,i}$, and standard

deviations, $\sigma_{\text{meas}, i}$, of measured quantities were represented by real list variables (Reichert, 1994).

The fitting procedure required three steps. First, the uncolonized IRE data was fit with a model containing only the diffusion process. Because there was no biofilm present, the diffusion ratio of the biofilm permeability to that of the bulk fluid, R , was set equal to one. This allowed the effective antibiotic diffusion coefficient to be equivalent to the calculated value for the diffusion coefficient of the antibiotic in water. Tabulated values for all variables used in the model can be found in Appendix A. The effective concentration boundary layer thickness, L_i , was allowed to vary in the fit. Once a fit was achieved, the estimated value of the appropriate boundary layer thickness for the uncolonized IRE was made constant and applied to all biofilm transport analyses.

The second step involved fitting the colonized IRE transport data with the diffusion model. In these fits the band area corresponding to the bulk fluid concentration was allowed to vary along with the diffusion coefficient of the antibiotic in water.

To further understand which transport processes were active in the biofilm, the third step was to fit the transport data with a model that accounted for the processes of diffusion, adsorption, and desorption. The first simulation of this model required varying the band area of the bulk fluid concentration, along with the rate constant variables, K_D and K_S , and the initial concentration of the

sorption particulate, S_o . Once a fit was established, the band area of the bulk fluid concentration was set constant and a new fit was run which allowed the diffusion coefficient of the antibiotic in water to vary, along with K_D , K_S , and S_o .

RESULTS

Planktonic Efficacy

All concentrations of levofloxacin were more efficacious against planktonic bacteria than ciprofloxacin (all p-values < 0.00001) (Table 1). Planktonic bacteria exposed to levofloxacin concentrations of 100, 250, and 500 $\mu\text{g ml}^{-1}$ for 30 minutes experienced log reductions of 4.26, 4.75, and 5.37, respectively, whereas the same doses of ciprofloxacin resulted in log reductions of 1.63, 2.16, and 1.97. A control experiment with no antibiotic produced a log reduction of -0.32 indicating that bacterial numbers increased during this time. Both antibiotics showed significant kill at all concentrations when compared to the control experiments (all p-values < 0.00001).

Table 1: Antibiotic efficacy against freely suspended bacteria.

Treatment ($\mu\text{g ml}^{-1}$)	Mean Log Reduction	Standard Deviation
Levo 100	4.26	0.148
Levo 250	4.75	0.248
Levo 500	5.37	0.212
Cipro 100	1.63	0.334
Cipro 250	2.15	0.119
Cipro 500	1.97	0.094
Control (no antibiotic)	-0.32	0.170

Colonization of IRE

Figure 9 shows typical bacterial spectra, from experiment 17, at various elapsed times. As bacterial cells attached to the IRE and colonized the surface, the IR adsorption bands increased in absorbance intensity. Monitoring the area of the Amide II band ($1580\text{--}1500\text{ cm}^{-1}$), which is indicative of bacterial protein, over time provided a "lateral" biofilm growth curve (Figure 10) characterizing the colonization of the IRE within approximately one micron of the surface.

Germanium IREs were colonized at rates varying between 0.05 and 0.40 Amide II band area units (abs cm^{-1}) per hour. Colonization curves from both sample chambers were nearly identical within each experiment; however, more variation in colonization rates occurred between experiments. These results are graphed and tabulated in Appendix C.

Biofilm Architecture

Inspection of the germanium coupons by confocal laser scanning microscopy showed that bacterial coverage was fairly confluent with 2-3 monolayers of bacteria at the thinnest sections (Figures 11 and 12). Z-section images portraying cross-sections of biofilm were used to estimate an average biofilm thickness of $15\text{ }\mu\text{m}$ varying from $5\text{ }\mu\text{m}$ at its thinnest to $25\text{ }\mu\text{m}$ in thicker sections (Figures 13 and 14). Fluorescent microscopic examination of

Biofilm Growth

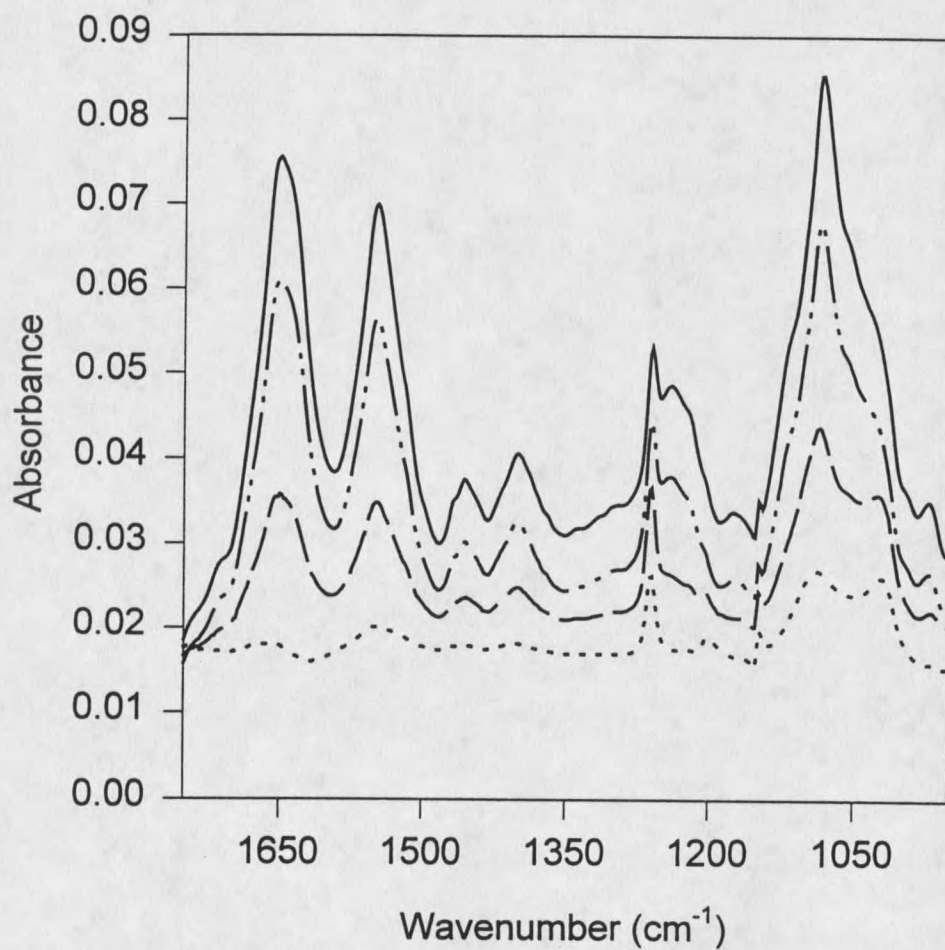


Figure 9: ATR-FTIR spectra of bacteria colonizing the IRE at 5 hrs (.....), 20 hrs (---), 30 hrs (-.-.-), and 40 hrs (—) for experiment #17. As more bacteria colonize the surface, the spectral bands increase in absorbance.

Lateral Growth Curve

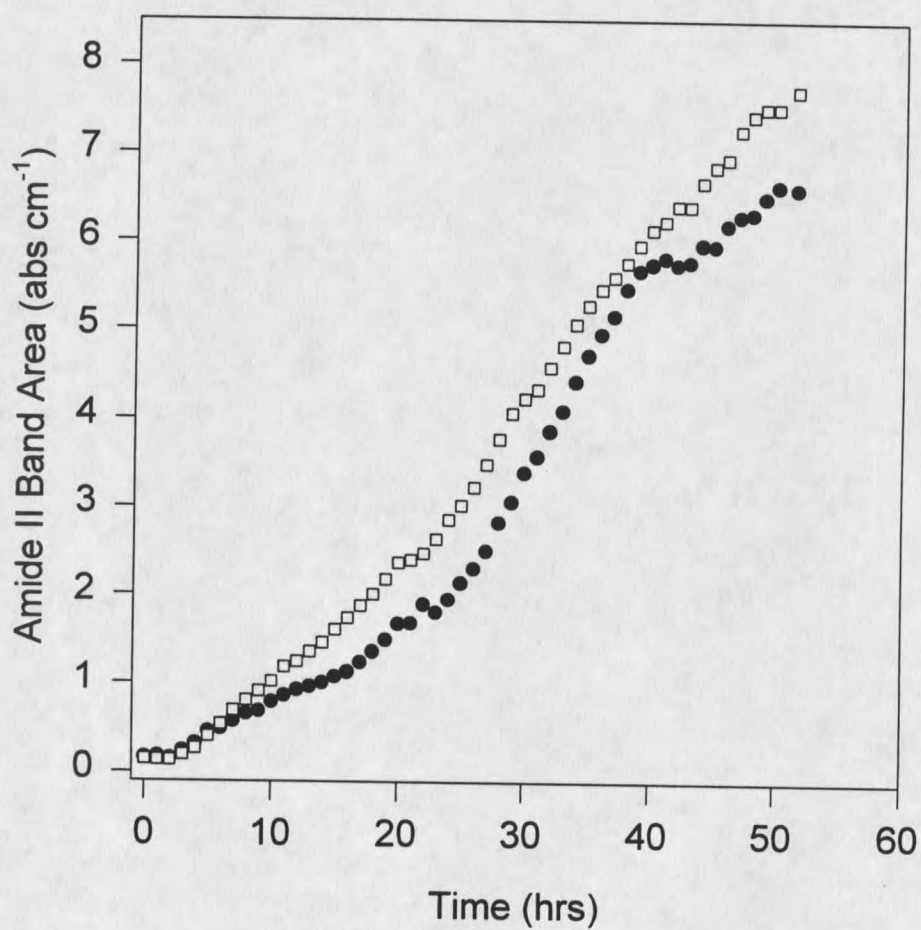


Figure 10: Lateral colonization of the IRE surface in sample chamber #1 (●) and sample chamber #2 (□) monitored by the area of the Amide II band ($1580\text{-}1500\text{ cm}^{-1}$) for experiment #18.

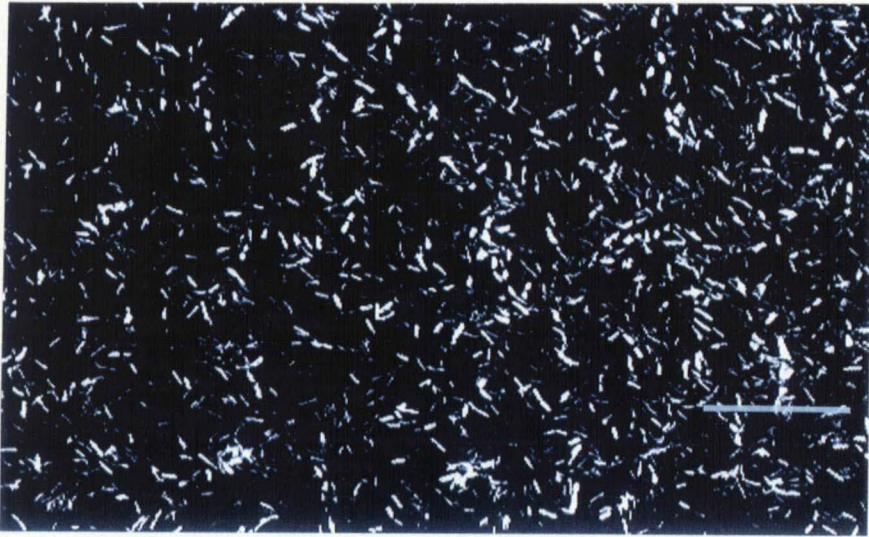


Figure 11: Confocal microscopy image (top view) of a relatively thin biofilm region. Bacteria are shown in white. The scale bar is 15 μm .

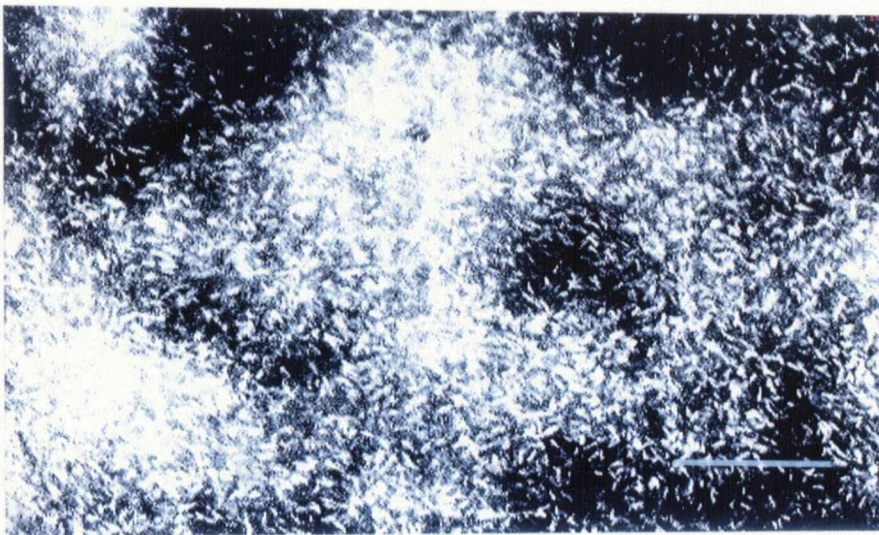


Figure 12: Confocal microscopy image (top view) of a thicker biofilm region. Bacteria are shown in white and gray. White indicates bacteria that were closer to the objective and gray indicates bacteria that were further away. The scale bar is 15 μm .

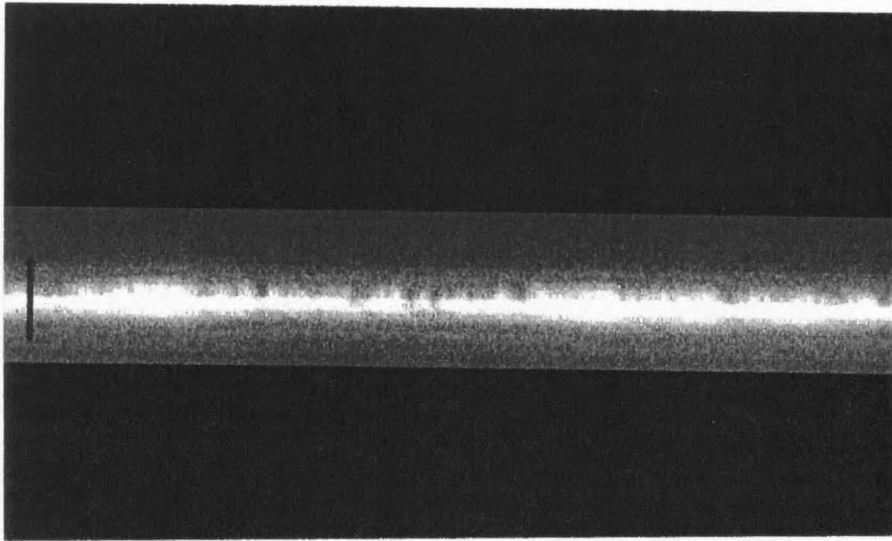


Figure 13: Confocal microscopy image (z-section) of a slice through a biofilm. The scale bar is 15 μm .

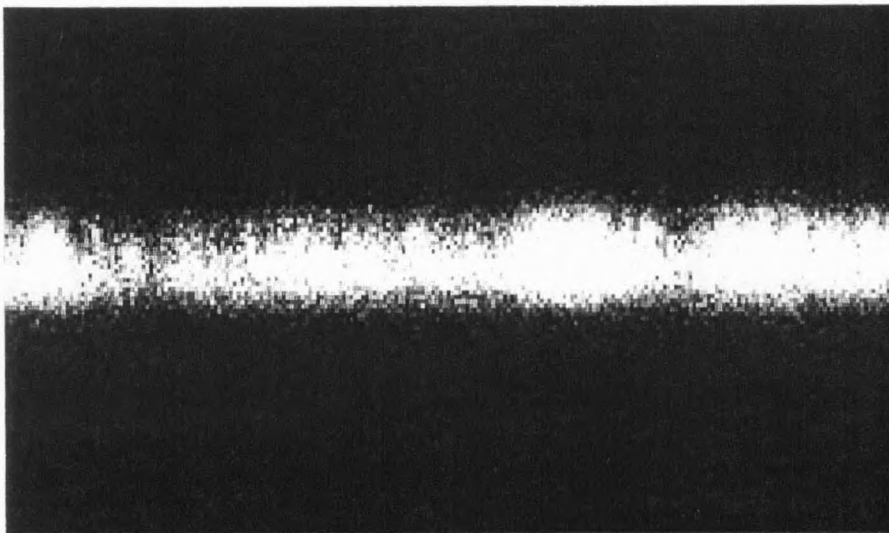


Figure 14: Close-up view of a confocal image (z-section) of a slice through a biofilm.

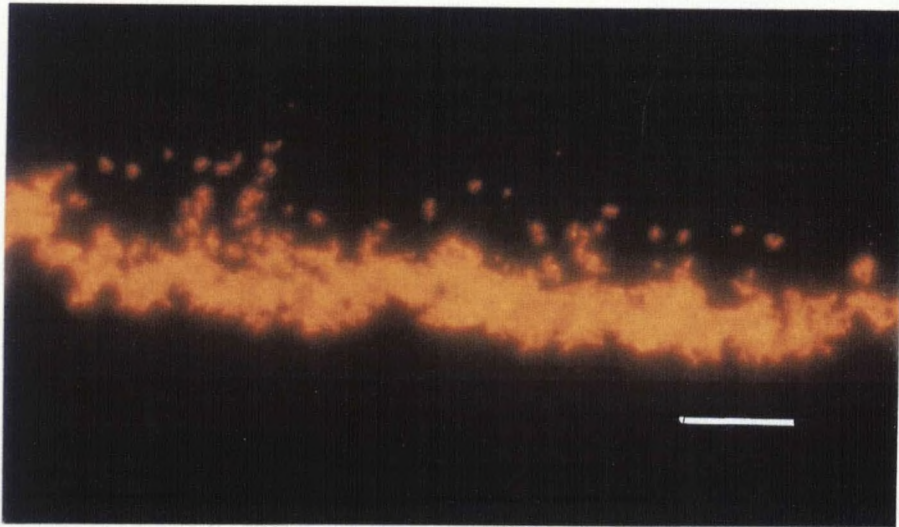


Figure 15: Image of a cryoembedded biofilm section stained with acridine orange. The scale bar is 20 μm .

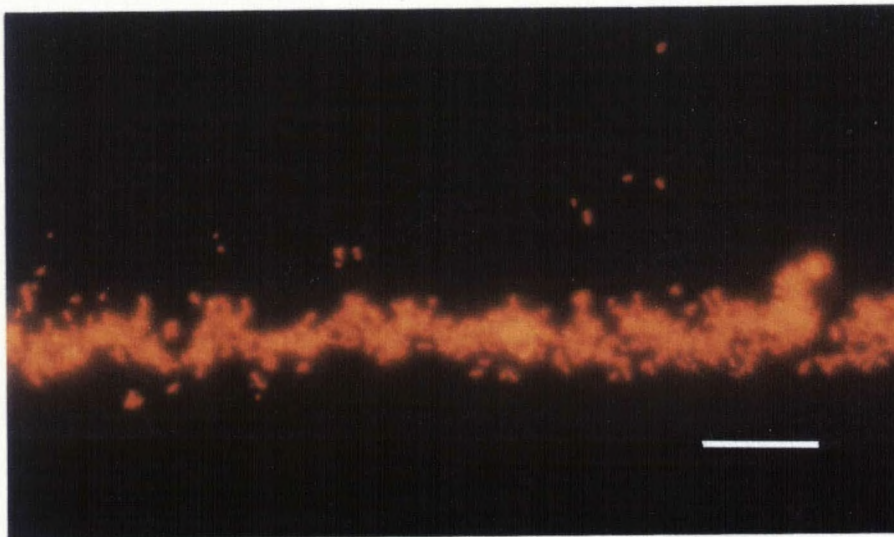


Figure 16: Image of a thinner cryoembedded biofilm section stained with acridine orange. The scale bar is 20 μm .

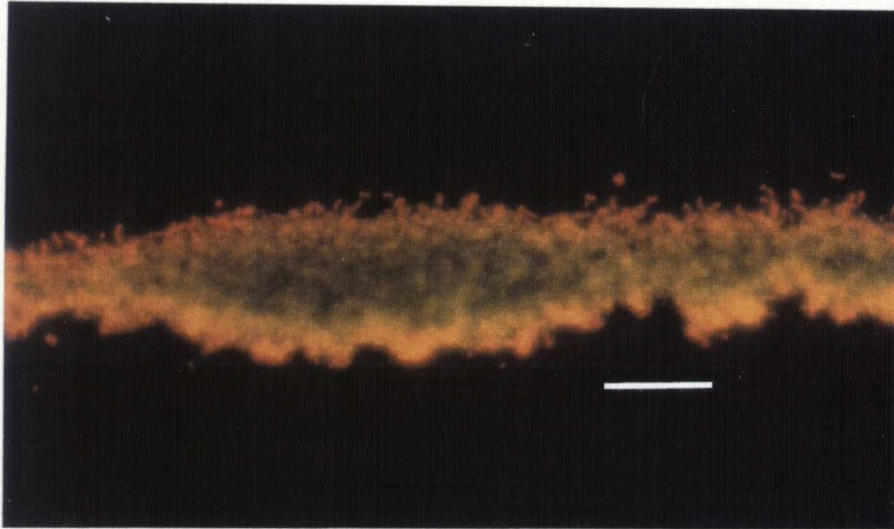


Figure 17: Image of a thicker, more dense cryoembedded biofilm section stained with acridine orange. The scale bar is 20 μm .



Figure 18: Image of a cryoembedded biofilm section stained with acridine orange. The scale bar is 20 μm .

cryoembedded biofilm sections showed a "loosely knit" biofilm (Figures 15 through 18) averaging $16.33 \pm 9.5 \mu\text{m}$ in thickness.

Biofilm Efficacy

Ciprofloxacin was slightly more efficacious against biofilm populations than levofloxacin but p-values showed no significant difference between the two antibiotics at concentrations of 100, 250, and $500 \mu\text{g ml}^{-1}$ ($p < 0.65$, $p < 0.18$, $p < 0.55$, respectively) (Table 2). Biofilms exposed to ciprofloxacin for 30 minutes at concentrations of 100, 250, and $500 \mu\text{g ml}^{-1}$ experienced a 1.14, 1.60, and 1.63 log reduction in the number of culturable cells, respectively. Biofilms exposed to levofloxacin at 100, 250, and $500 \mu\text{g ml}^{-1}$ had log reductions of 0.98, 1.09, and 1.42, respectively. The control experiment had a log reduction of -0.025 indicating no change in cell numbers. Both antibiotics showed significant kill when compared to the control experiments. The p-values for levofloxacin at concentrations of 100, 250, and $500 \mu\text{g ml}^{-1}$ were $p < 0.0185$, $p < 0.0120$, and $p < 0.0035$, respectively. For the same concentrations of ciprofloxacin, p-values were $p < 0.0100$, $p < 0.0020$, and $p < 0.0020$. Biofilm efficacy was less than planktonic efficacy in all cases. Results comparing levofloxacin and ciprofloxacin treatments against planktonic and biofilm populations are graphed in Figure 19.

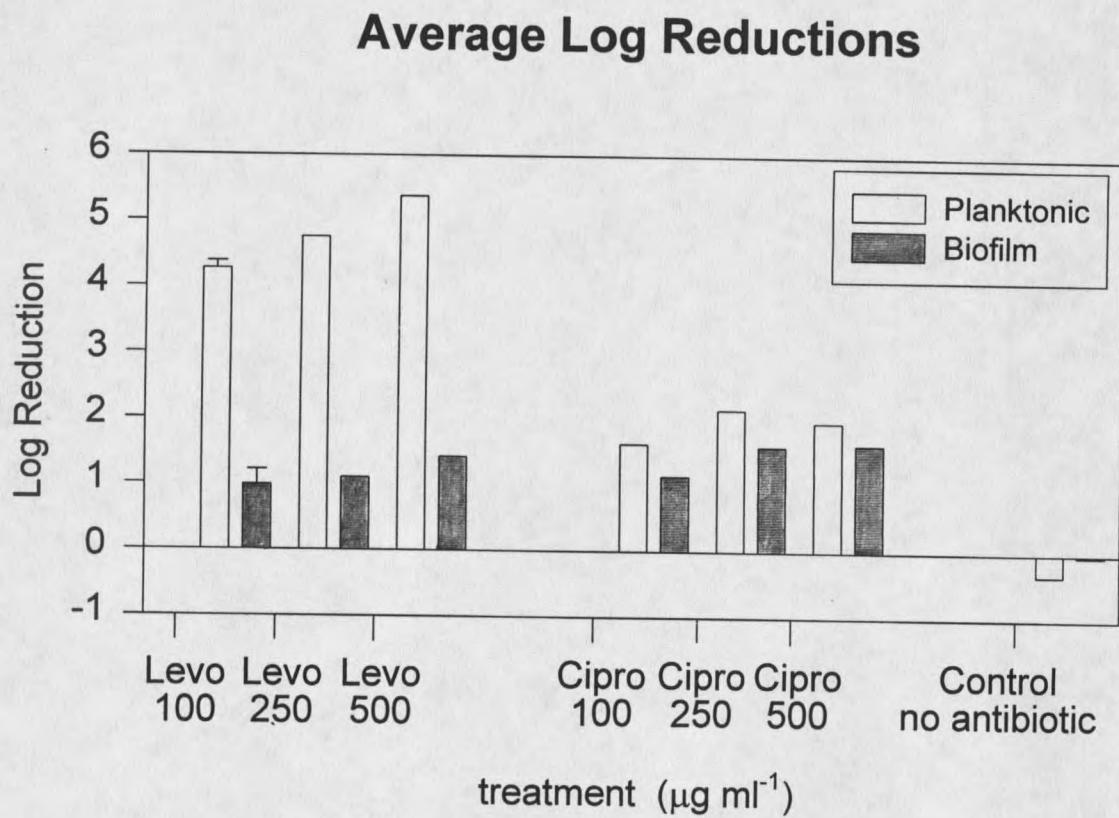


Figure 19: Antibiotic efficacy of levofloxacin and ciprofloxacin against planktonic and biofilm organisms.

Table 2: Antibiotic efficacy against biofilm bacteria.

Treatment ($\mu\text{g ml}^{-1}$)	Mean Log Reduction	Standard Deviation
Levo 100	0.98	0.489
Levo 250	1.09	0.611
Levo 500	1.42	0.104
Cipro 100	1.14	0.117
Cipro 250	1.60	0.307
Cipro 500	1.63	0.096
Control (no antibiotic)	-0.03	0.054

Antibiotic transport to substratum (FTIR)

Uncolonized IRE Experiments

From the sampled FTIR spectra, areas of ciprofloxacin band $1350\text{-}1325\text{ cm}^{-1}$ and levofloxacin band $995\text{-}965\text{ cm}^{-1}$ were monitored over time to produce the antibiotic delivery curves shown in Figures 20 through 25. These bands were chosen because they increased linearly in absorbance with respect to concentration and because they appeared in regions that were relatively free from bacterial interference. In the uncolonized IRE experiments (no biofilm), the concentration of levofloxacin rose to a plateau level within the first 70 to 150 seconds after introduction to the FTIR chamber. All concentrations maintained a

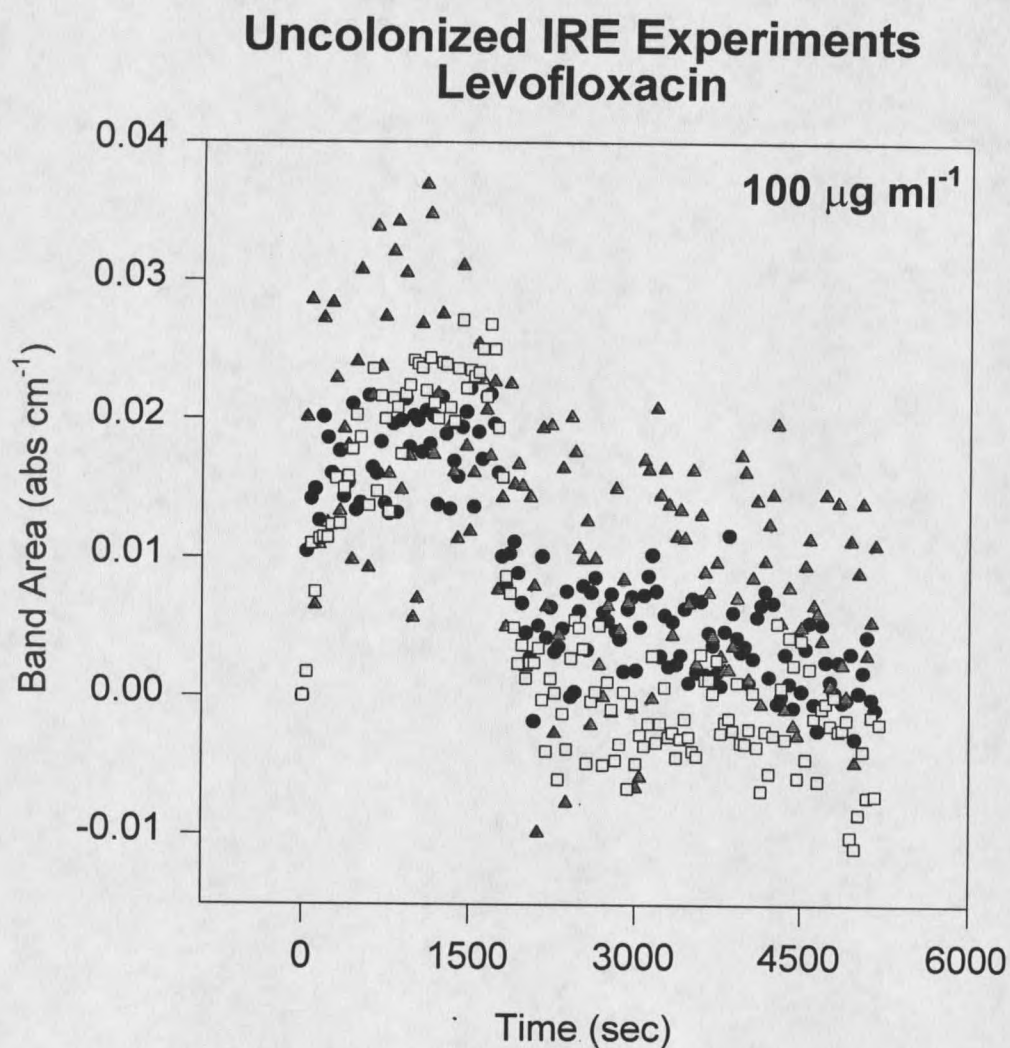


Figure 20: Levofloxacin delivery data ($100 \mu\text{g ml}^{-1}$) for uncolonized IRE experiments #22 (●), #25 (□), and #28 (▲). Areas of the band $995\text{-}965 \text{ cm}^{-1}$ were plotted versus time in seconds.

Uncolonized IRE Experiments Levofloxacin

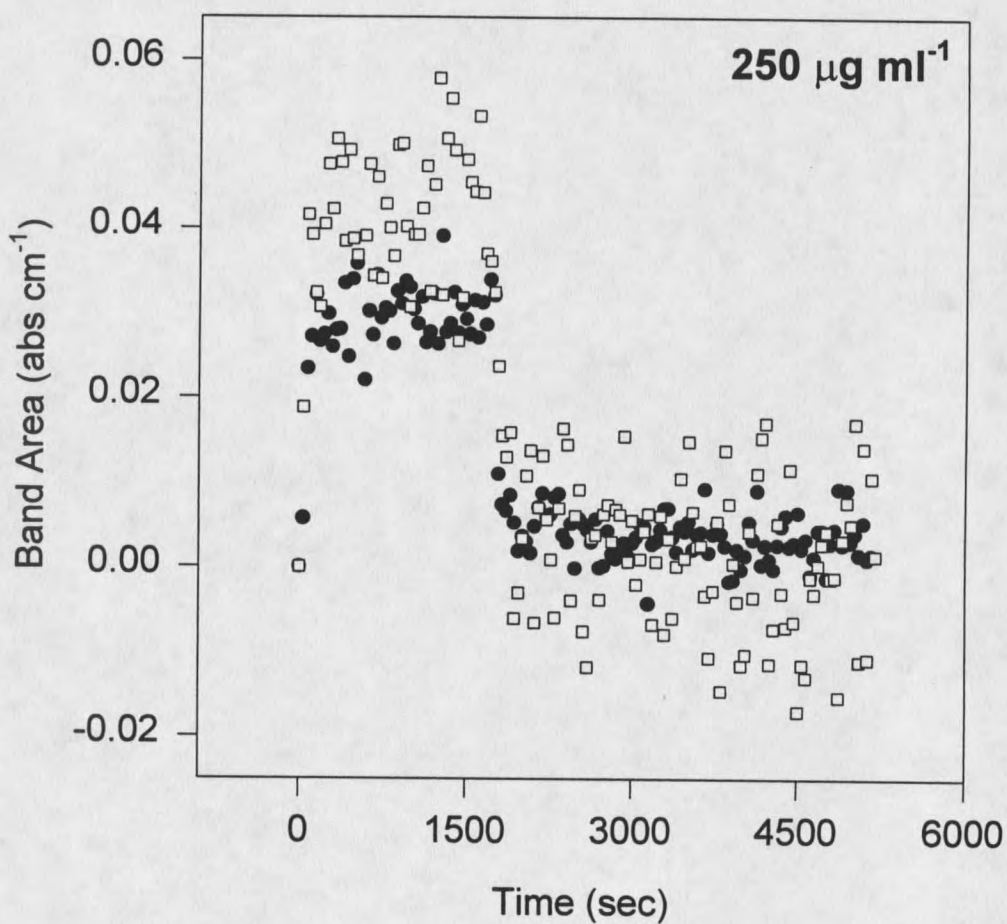


Figure 21: Levofloxacin delivery data (250 $\mu\text{g ml}^{-1}$) for uncolonized IRE experiments #23 (●) and #26 (□). Areas of the band 995-965 cm^{-1} were plotted versus time in seconds.

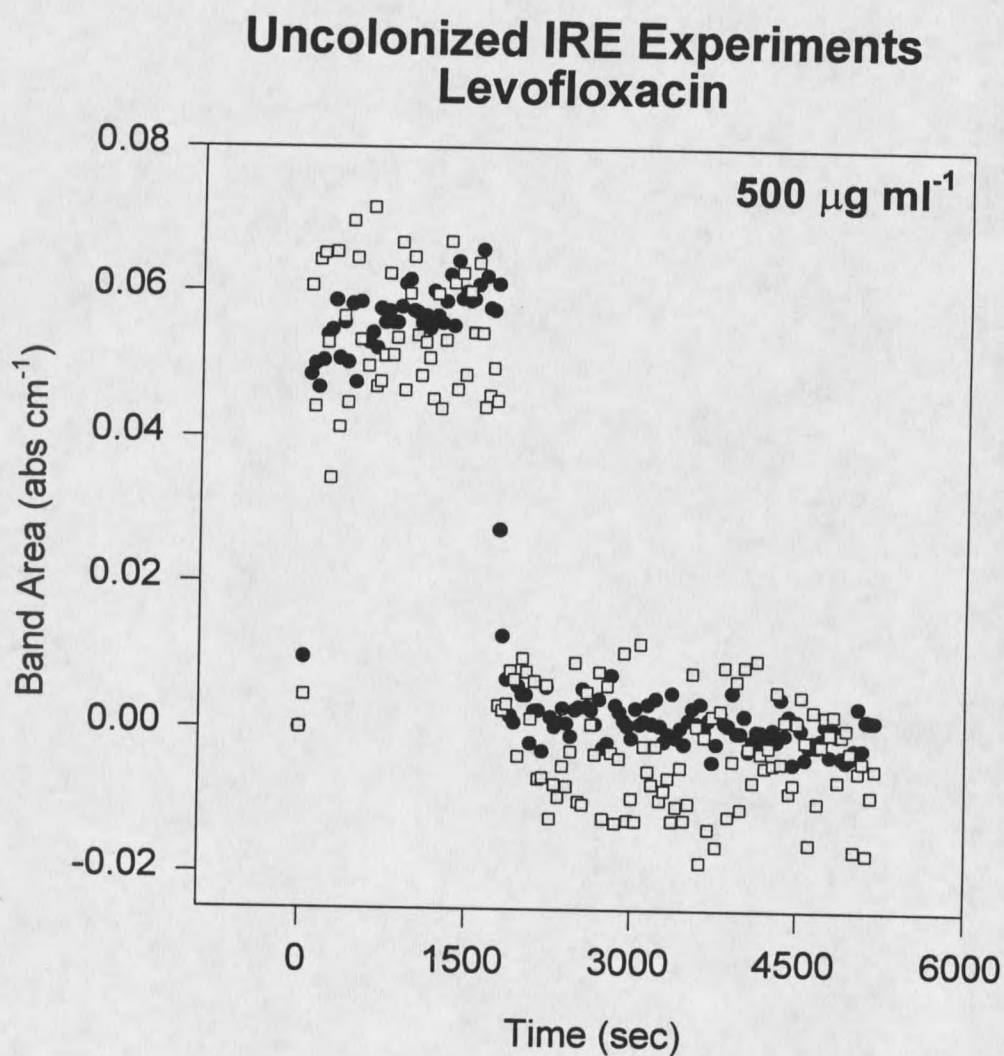


Figure 22: Levofloxacin delivery data (500 $\mu\text{g ml}^{-1}$) for uncolonized IRE experiments #24 (●) and #27 (□). Areas of the band 995-965 cm^{-1} were plotted versus time in seconds.

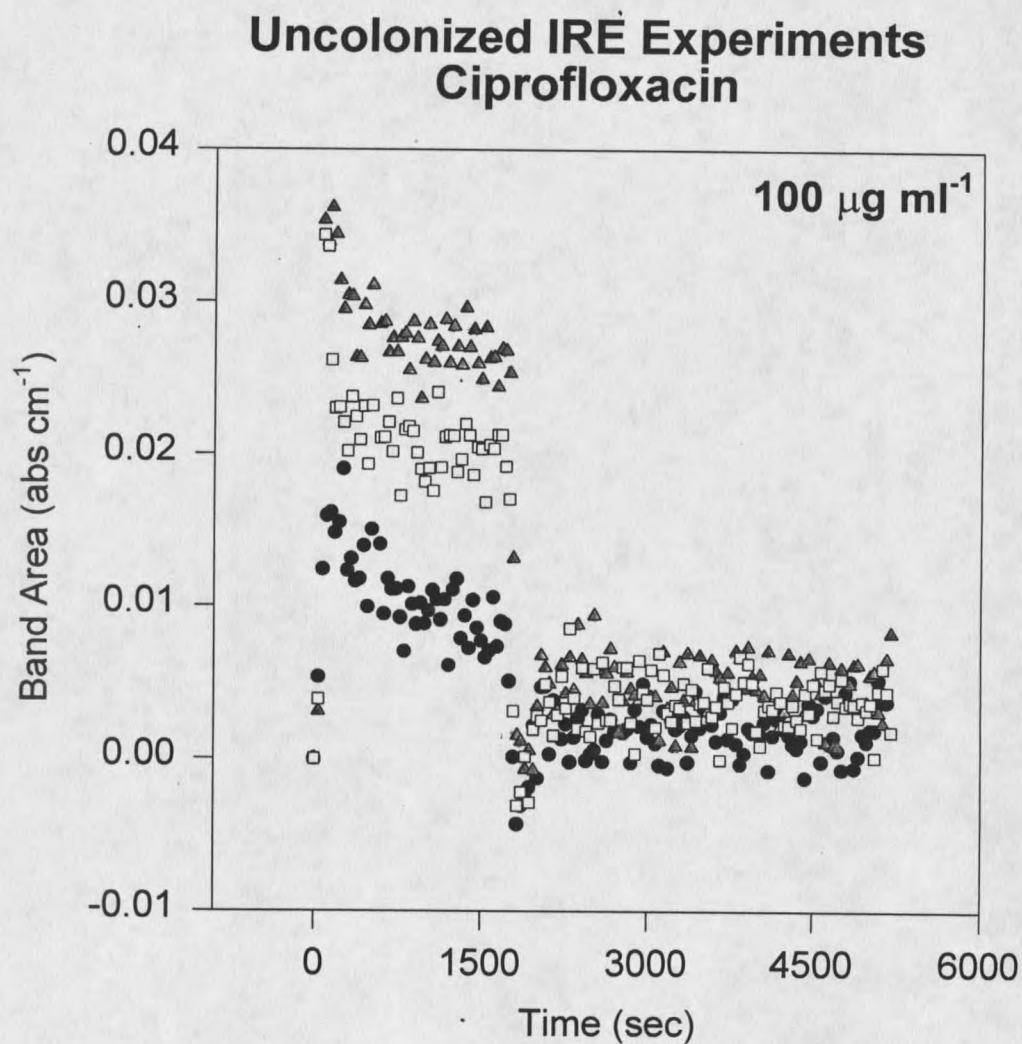


Figure 23: Ciprofloxacin delivery data ($100 \mu\text{g ml}^{-1}$) for uncolonized IRE experiments #22 (●), #25 (□), and #28 (▲). Areas of the band $1350\text{-}1325 \text{ cm}^{-1}$ were plotted versus time in seconds.

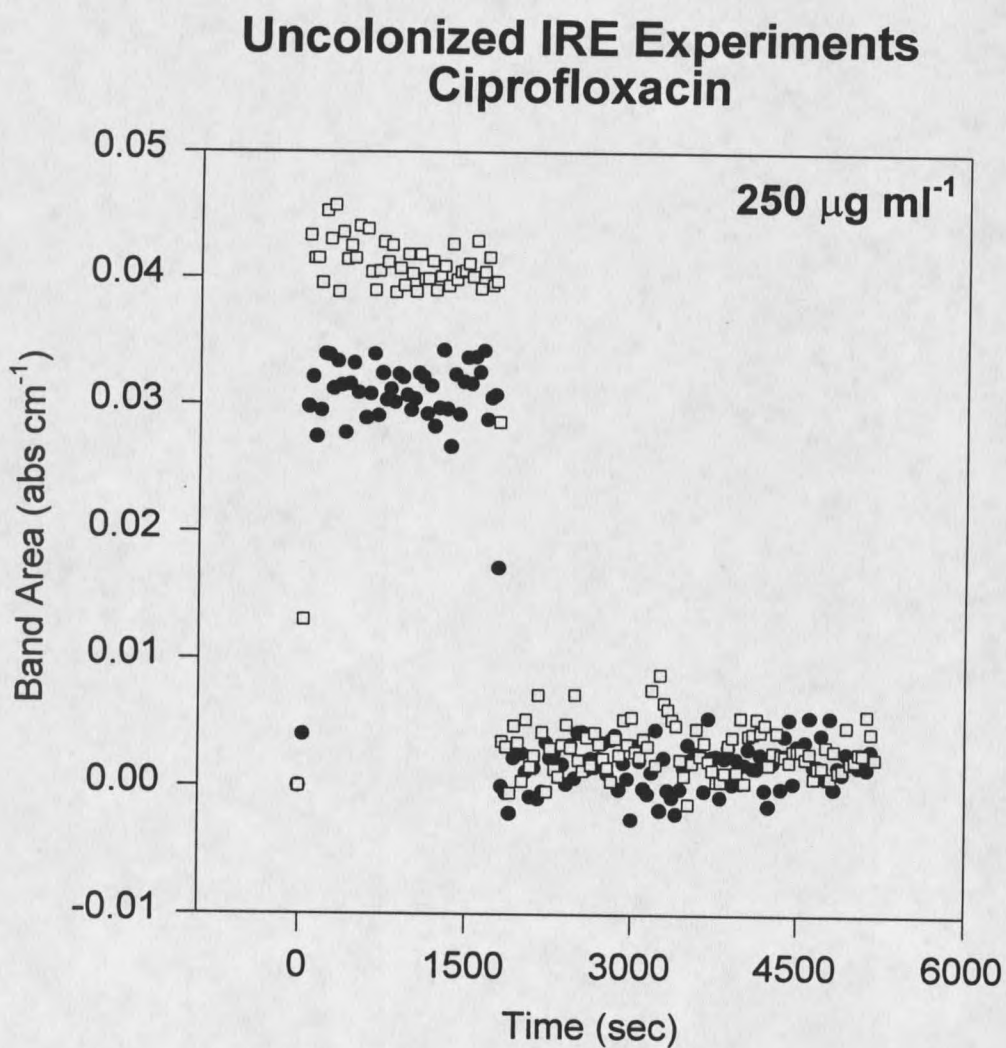


Figure 24: Ciprofloxacin delivery data (250 $\mu\text{g ml}^{-1}$) for uncolonized IRE experiments #23 (●) and #26 (□). Areas of the band 1350-1325 cm⁻¹ were plotted versus time in seconds.

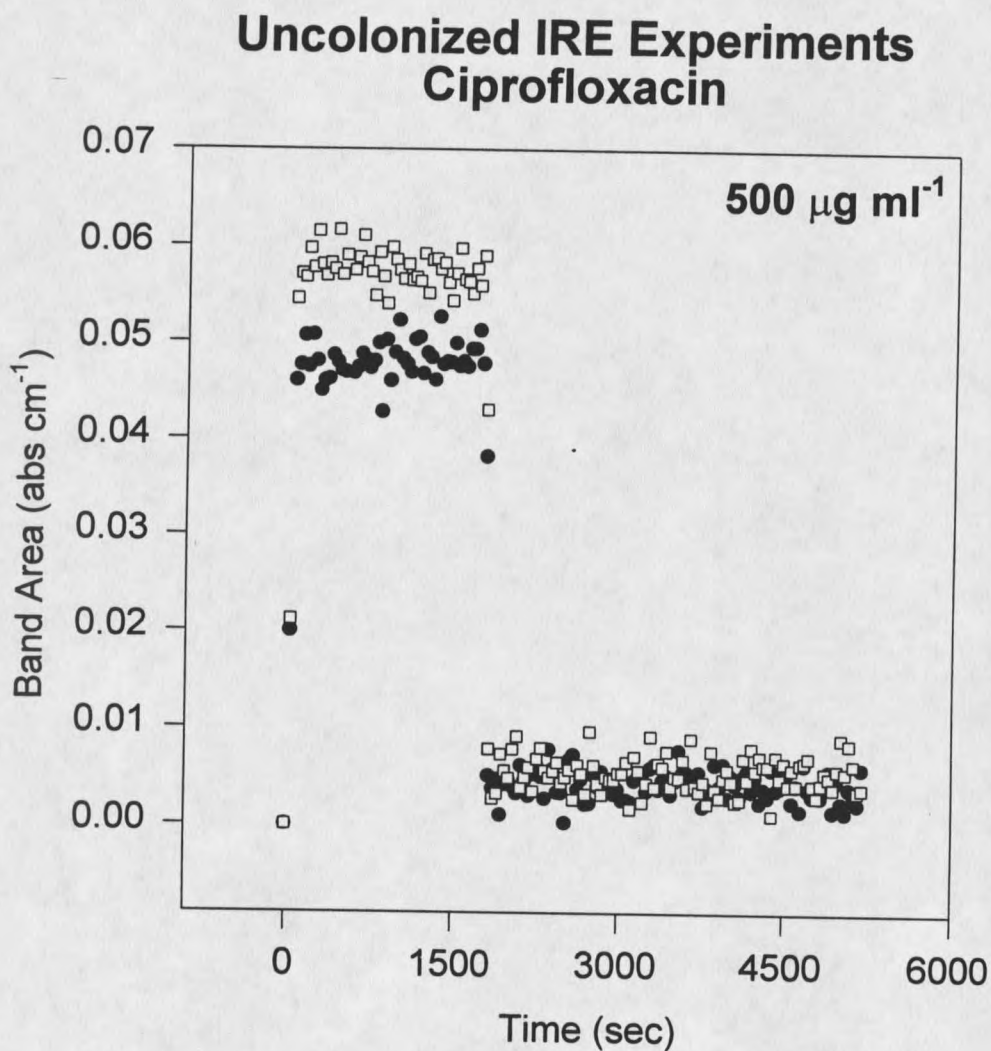


Figure 25: Ciprofloxacin delivery data ($500 \mu\text{g ml}^{-1}$) for uncolonized IRE experiments #24 (●) and #27 (□). Areas of the band $1350\text{-}1325 \text{ cm}^{-1}$ were plotted versus time in seconds.

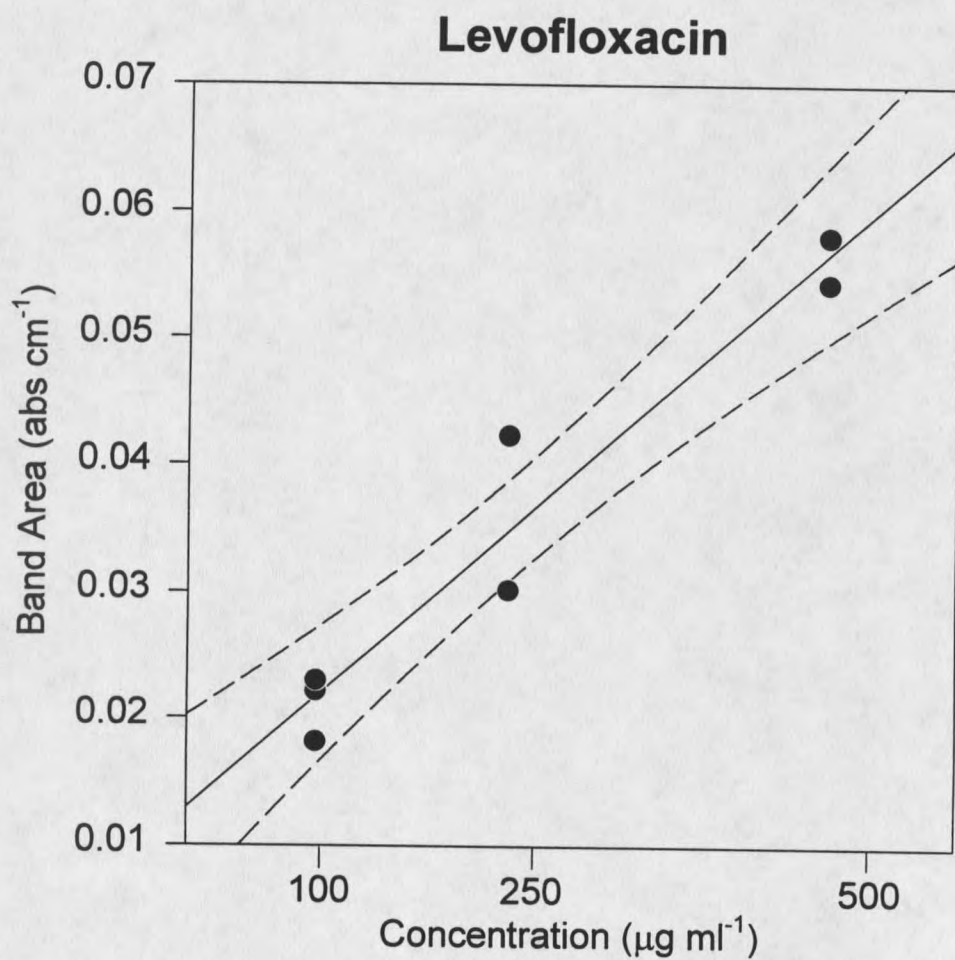


Figure 26: Calibration curve for levofloxacin relating the area of band 995-965 cm⁻¹ to concentration. Experimental data (●), a linear regression (—), and the 95% confidence interval (---) are shown.

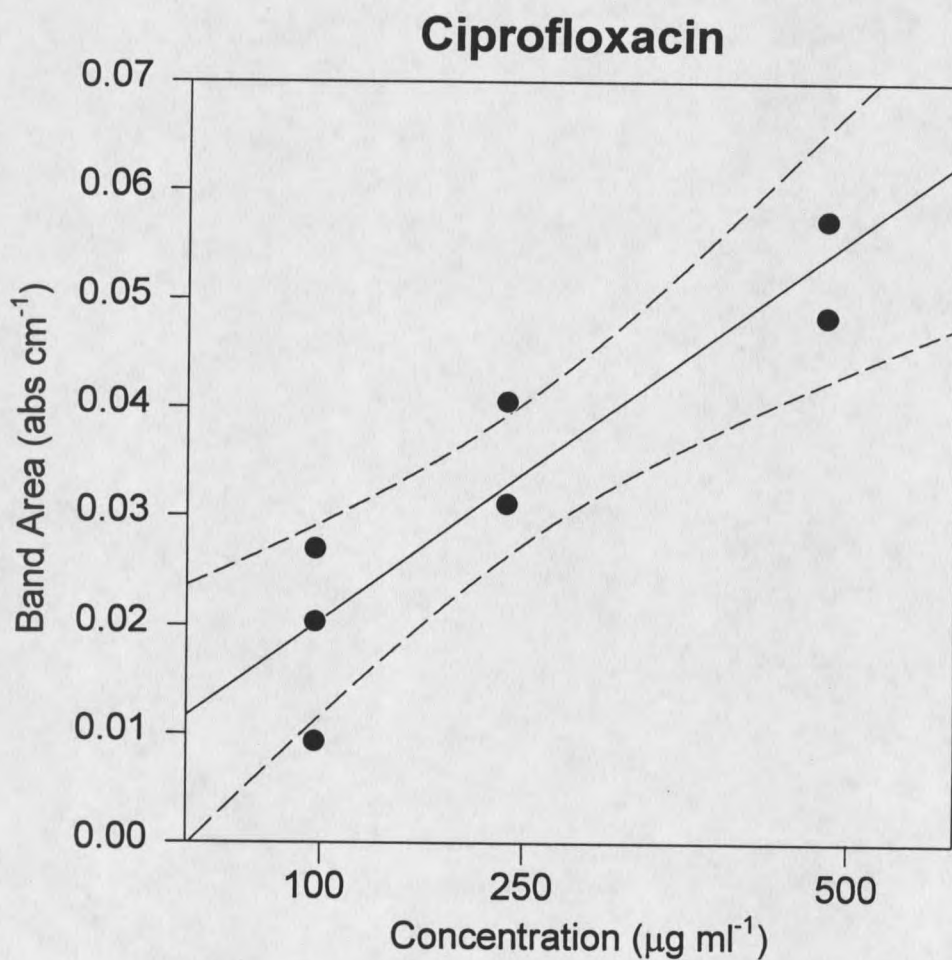


Figure 27: Calibration curve for ciprofloxacin relating the area of band 1350-1325 cm⁻¹ to concentration. Experimental data (●), a linear regression (—), and the 95% confidence interval (---) are shown.

plateau during the 30 minute antibiotic dose. Average areas of band 995-965 cm^{-1} for the plateau regions at 100, 250, and 500 $\mu\text{g ml}^{-1}$ concentrations were approximately 0.0200, 0.0350, and 0.0525 abs cm^{-1} , respectively (Figures 20 through 22). At 30 minutes, upon initiation of the rinse, band areas for all concentrations dropped immediately to zero indicating that the antibiotic was immediately washed out of the sample chamber.

The ciprofloxacin delivery curves had the same step-function shape as the levofloxacin curves: immediate rise, plateau, immediate drop. Ciprofloxacin reached its plateau level in approximately the first 70 to 100 seconds, slightly quicker than levofloxacin. The average areas of band 1350-1325 cm^{-1} for the plateau regions at concentrations of 100, 250, and 500 $\mu\text{g ml}^{-1}$ were 0.0200, 0.0350, and 0.0550 abs cm^{-1} , respectively (Figures 23 through 25).

Band area values from the last 20 minutes of antibiotic delivery, immediately preceding the rinse, were averaged and plotted versus concentration to obtain the calibration curves shown in Figures 26 and 27. Values from these calibration curves were used in modeling the antibiotic penetration of biofilms and to interpret the colonized IRE transport curves.

Colonized IRE Experiments

Spectra of levofloxacin and ciprofloxacin sampled at 5, 10, and 25 minutes during antibiotic delivery to the substratum are shown in Figures 28 and 29,

Levofloxacin Delivery

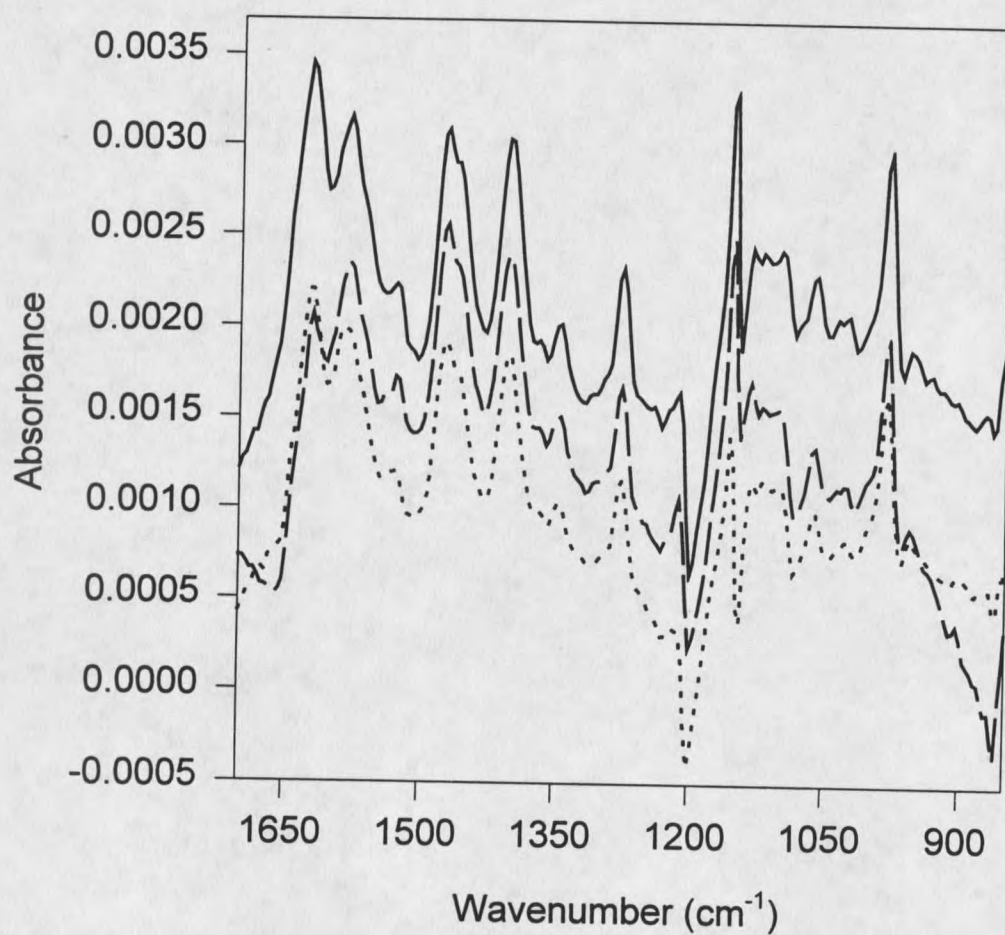


Figure 28: ATR-FTIR spectra of levofloxacin (250 $\mu\text{g ml}^{-1}$) delivery to a colonized IRE for experiment #17. Spectra shown were sampled at 5 min (.....), 10 min (---), and 25 min (—). As more antibiotic accumulated at the interface, the spectral bands increased in absorbance.

Ciprofloxacin Delivery

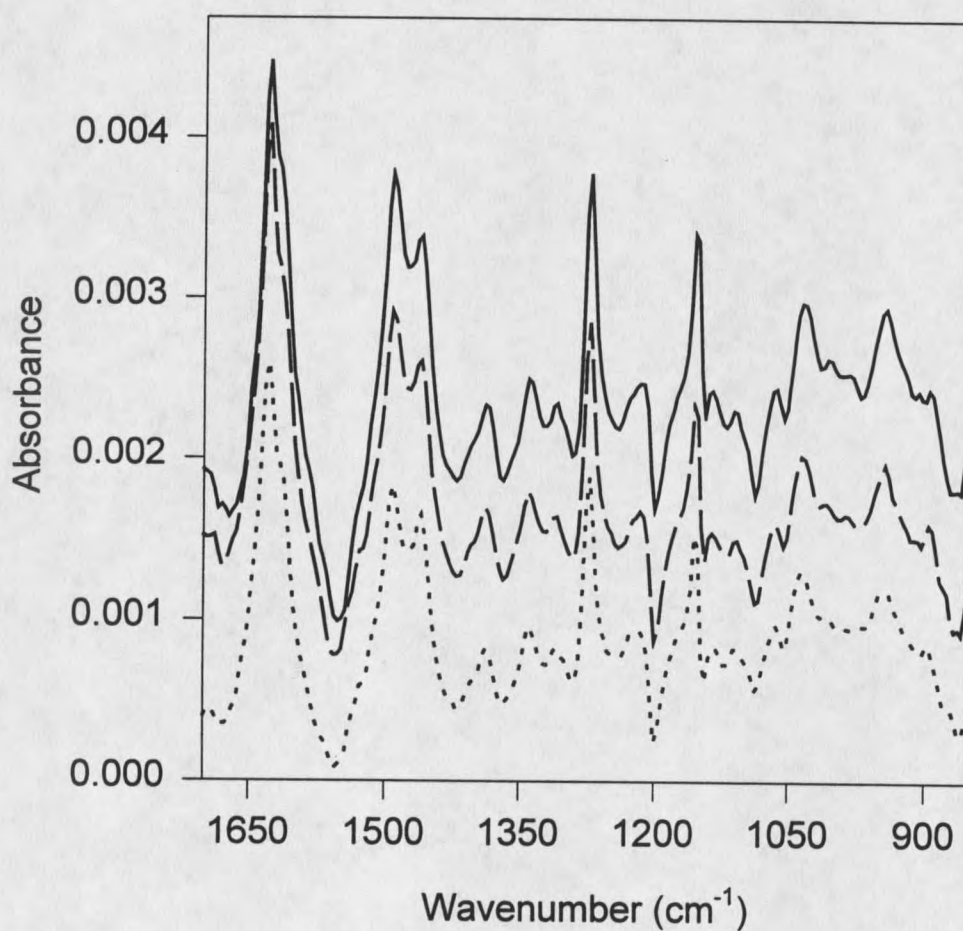


Figure 29: ATR-FTIR spectra of ciprofloxacin ($250 \mu\text{g ml}^{-1}$) delivery to a colonized IRE for experiment #17. Spectra shown were sampled at 5 min (.....), 10 min (---), and 25 min (—). As more antibiotic accumulated at the interface, the spectral bands increased in absorbance.

Colonized IRE Experiments Levofloxacin

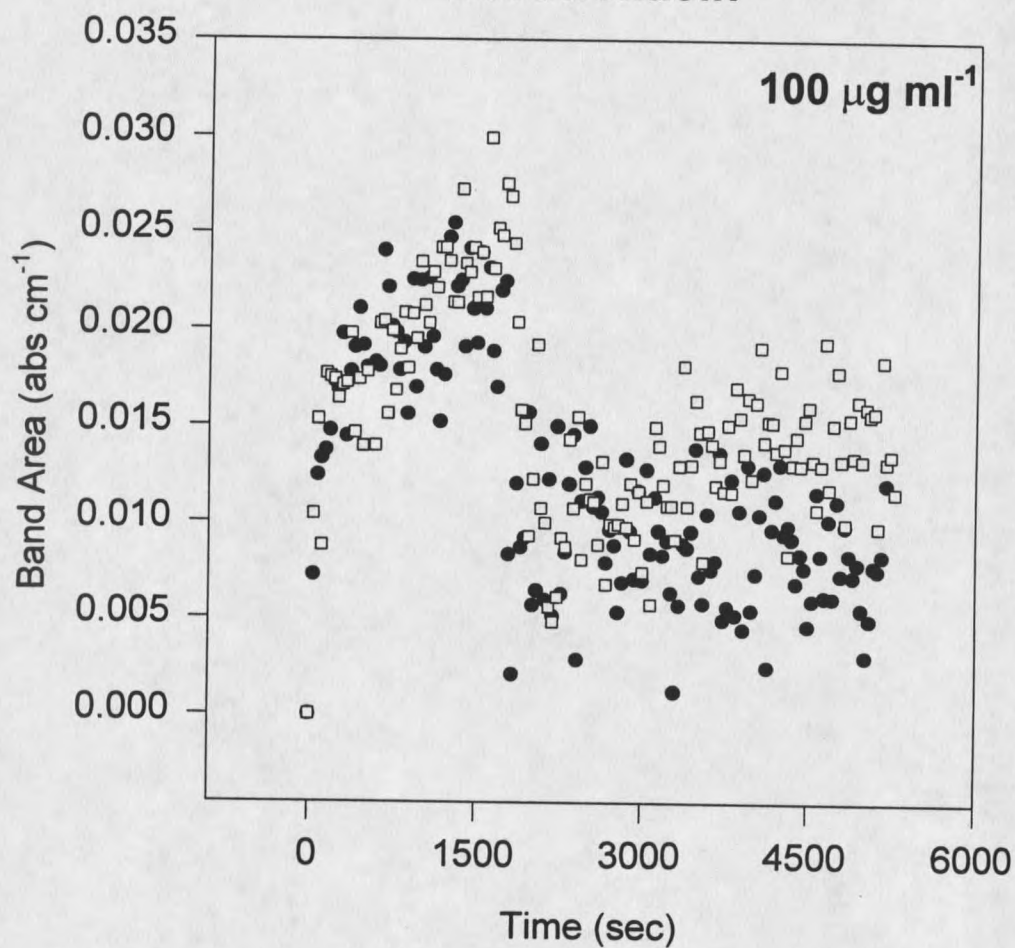


Figure 30: Levofloxacin delivery data (100 µg ml⁻¹) for colonized IRE experiments #16 (●) and #19 (□). Areas of the band 995-965 cm⁻¹ were plotted versus time in seconds.

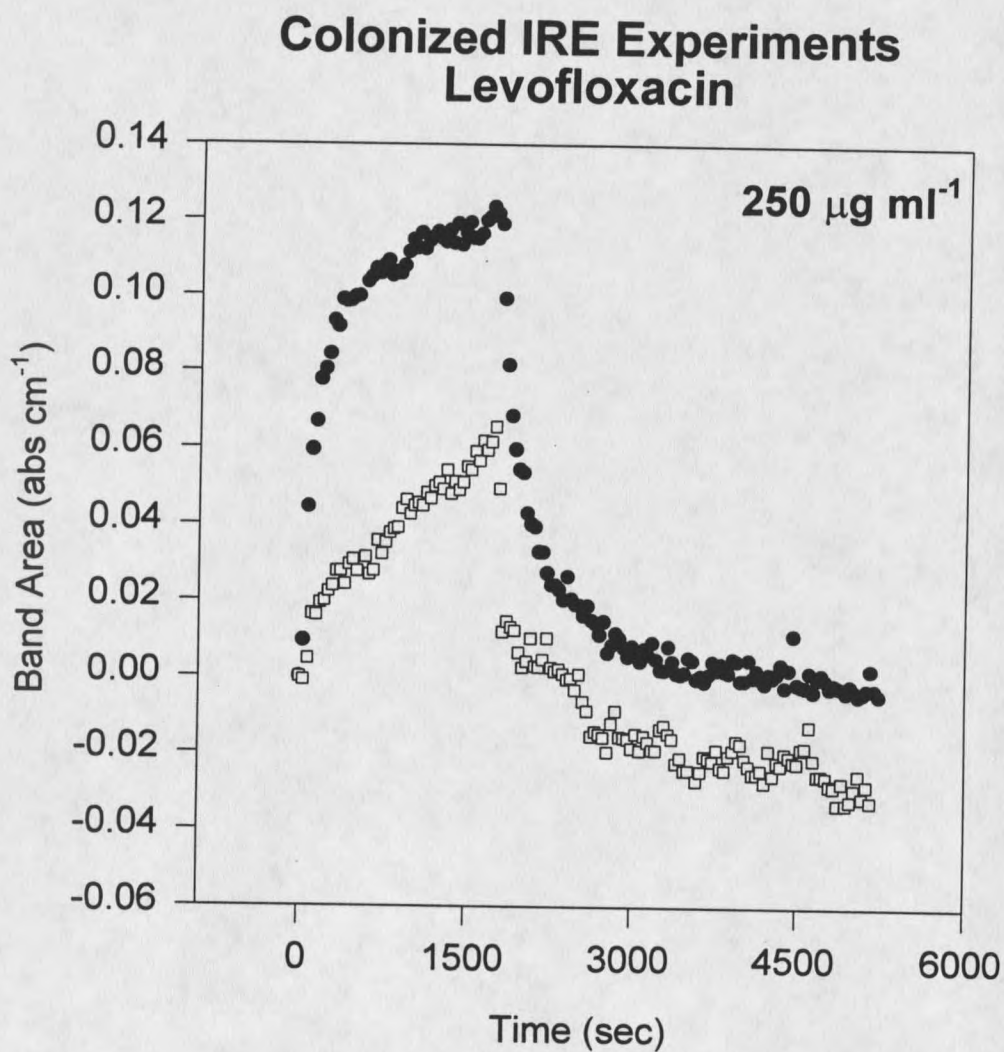


Figure 31: Levofloxacin delivery data ($250 \mu\text{g ml}^{-1}$) for colonized IRE experiments #17 (●) and #20 (□). Areas of the band $995\text{-}965 \text{ cm}^{-1}$ were plotted versus time in seconds. Experiment #20 is erroneous due to a weak FTIR signal surrounding the band of interest.

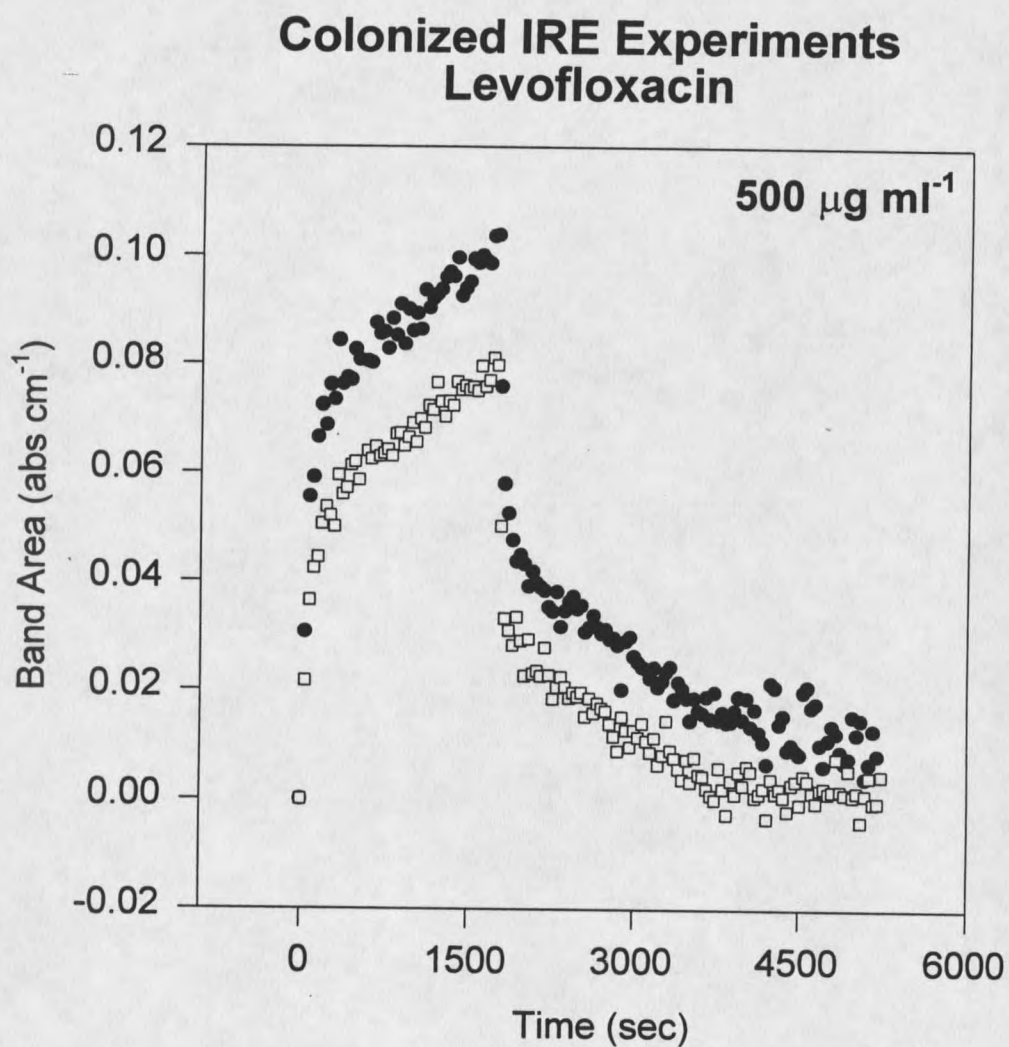


Figure 32: Levofloxacin delivery data ($500 \mu\text{g ml}^{-1}$) for colonized IRE experiments #15 (●) and #18 (□). Areas of the band $995\text{-}965 \text{ cm}^{-1}$ were plotted versus time in seconds.

Colonized IRE Experiments Ciprofloxacin

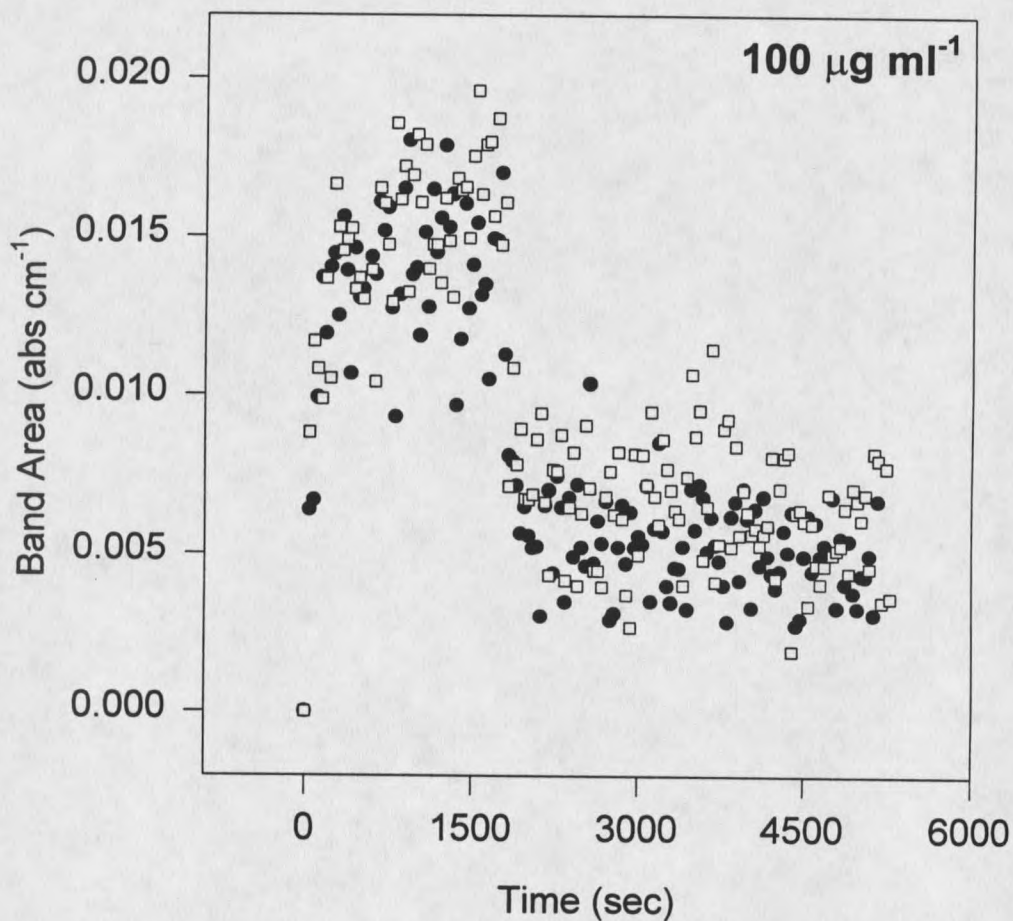


Figure 33: Ciprofloxacin delivery data (100 µg ml⁻¹) for colonized IRE experiments #16 (●) and #19 (□). Areas of the band 1350-1325 cm⁻¹ were plotted versus time in seconds.

Colonized IRE Experiments Ciprofloxacin

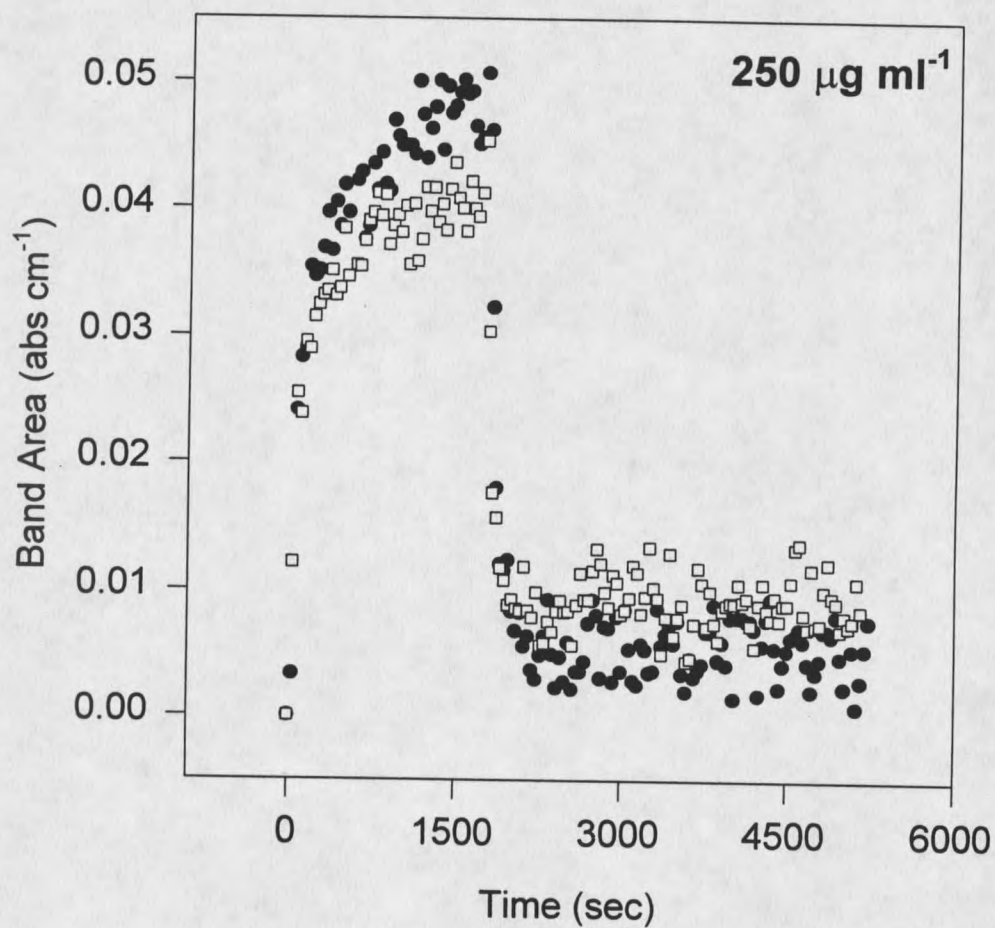


Figure 34: Ciprofloxacin delivery data ($250 \mu\text{g ml}^{-1}$) for colonized IRE experiments #17 (●) and #20 (□). Areas of the band $1350\text{-}1325 \text{ cm}^{-1}$ were plotted versus time in seconds.

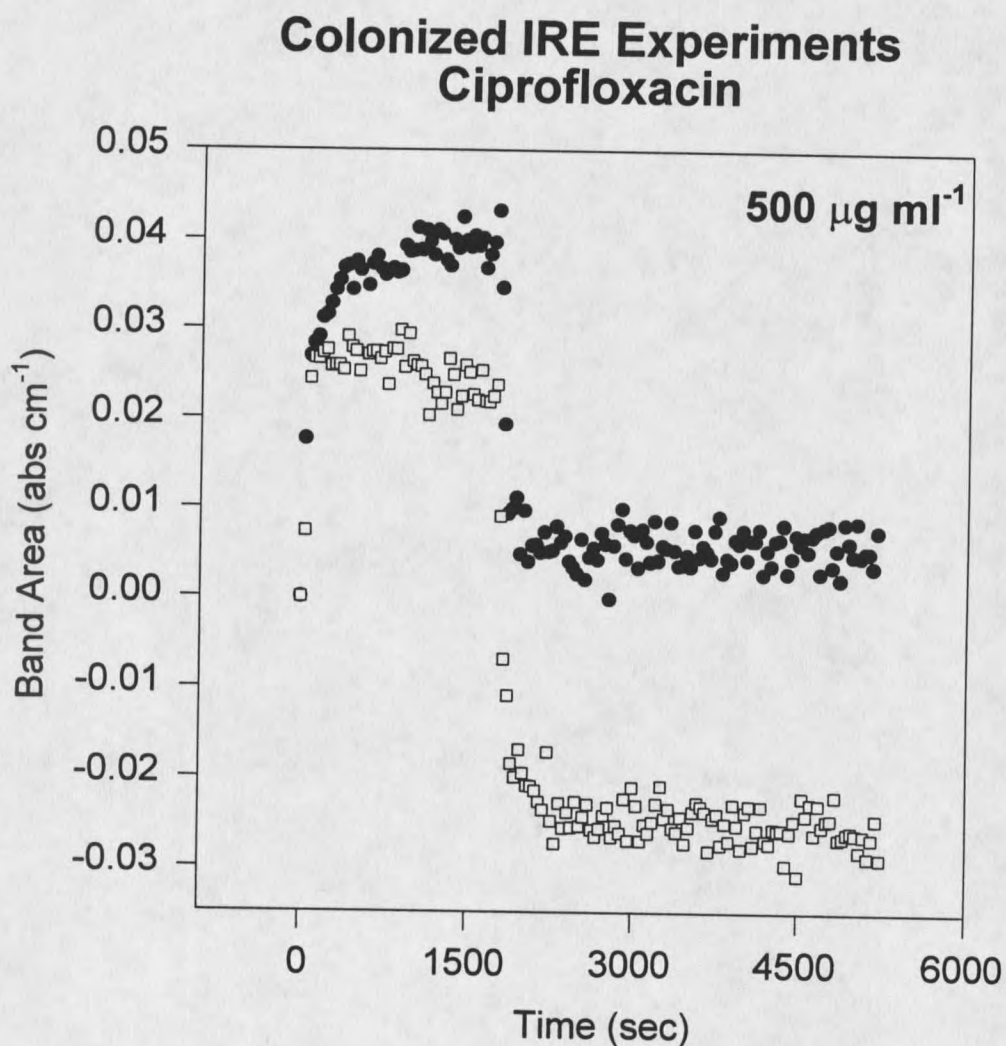


Figure 35: Ciprofloxacin delivery data ($500 \mu\text{g ml}^{-1}$) for colonized IRE experiments #15 (●) and #18 (□). Areas of the band $1350\text{-}1325 \text{ cm}^{-1}$ were plotted versus time in seconds. Experiment #18 is erroneous due to a spike in the FTIR spectra which interfered with the band of interest.

respectively. The areas of levofloxacin band $995\text{-}965\text{ cm}^{-1}$ and ciprofloxacin band $1350\text{-}1325\text{ cm}^{-1}$ were monitored over time to obtain delivery curves.

Figures 30 through 35 illustrate duplicate biofilm experiments for the deliveries of levofloxacin and ciprofloxacin at 100 , 250 , and $500\text{ }\mu\text{g ml}^{-1}$. The shapes of the antibiotic delivery curves for the colonized IREs differed from those of the uncolonized IREs.

For levofloxacin, the delivery curve from experiment 20 was erroneous due to a weak FTIR spectra in the area surrounding the band of interest ($995\text{-}965\text{ cm}^{-1}$). Disregarding this one experiment, within the first 5 minutes of antibiotic treatment the concentration of levofloxacin at the substratum was found to be at least 78% of the calibrated bulk fluid value (Table 3). The error range for this estimate is shown by the percentage of the low and high confidence interval for bulk fluid concentration reached in 5 minutes. In half of the experiments, the concentration of levofloxacin at the substratum exceeded that of the bulk fluid after 5 minutes. By 30 minutes, the bulk fluid concentration had been attained or exceeded by all levofloxacin experiments (Table 4). All experiments exhibited a rapid rise in the substratum concentration within the first 3 minutes of antibiotic delivery, followed by a gradual increase over the remaining 30 minute treatment. Upon initiation of the rinse, the levofloxacin concentration dropped sharply in the first 2 minutes, then washed out gradually. The antibiotic concentration at the substratum returned to zero by the end of the 90 minute rinse.

Table 3: Penetration of levofloxacin after 5 min based on percentages of the calibrated bulk fluid concentration and the low and high confidence interval limits of the bulk fluid concentration. * indicates erroneous data.

Experiment	Treatment ($\mu\text{g ml}^{-1}$)	% Calib. Bulk	% Low C.I.	% High C.I.
16	Levo 100	79.07	113.33	68.00
19	Levo 100	79.07	113.33	68.00
17	Levo 250	281.25	300.00	236.84
20*	Levo 250	78.13	83.33	65.79
15	Levo 500	140.00	160.42	123.20
18	Levo 500	100.00	114.58	88.00

Table 4: Penetration of levofloxacin after 30 min based on percentages of the calibrated bulk fluid concentration and the low and high confidence interval limits of the bulk fluid concentration. * indicates erroneous data.

Experiment	Treatment ($\mu\text{g ml}^{-1}$)	% Calib. Bulk	% Low C.I.	% High C.I.
16	Levo 100	102.33	146.67	88.00
19	Levo 100	125.58	180.00	108.00
17	Levo 250	381.25	406.67	321.05
20*	Levo 250	203.13	216.67	171.05
15	Levo 500	189.09	216.67	166.40
18	Levo 500	145.45	166.67	128.00

Similar characteristics were exhibited by the ciprofloxacin curves when a biofilm was present. The delivery curve from experiment 18 was also considered erroneous due to a glitch in the FTIR spectrum that interfered with the band of interest ($1350\text{-}1325\text{ cm}^{-1}$). As Table 5 shows, the ciprofloxacin concentration at the substratum rapidly reached at least 65% of the bulk fluid concentration

(omitting the erroneous data) within the first 5 minutes of being introduced to the sample chamber. At 30 minutes, the substratum concentration was at least 78% of the bulk fluid (Table 6). All experiments exhibited a rapid rise in the substratum concentration within the first 3 minutes of antibiotic delivery, followed by a gradual increase over the remaining 30 minute treatment. Upon initiation of the washout rinse, the substratum concentration quickly dropped to a band area level of $0.005 \text{ abs cm}^{-1}$ and maintained this level for the duration of the experiment.

Table 5: Penetration of ciprofloxacin after 5 min based on percentages of the calibrated bulk fluid concentration and the low and high confidence interval limits of the bulk fluid concentration. * indicates erroneous data.

Exp.	Treatment ($\mu\text{g ml}^{-1}$)	% Calib. Bulk	% Low C.I.	% High C.I.
16	Cipro 100	76.32	145.00	48.33
19	Cipro 100	73.68	140.00	46.67
17	Cipro 250	115.38	150.00	98.68
20	Cipro 250	101.54	132.00	86.84
15	Cipro 500	64.81	83.33	53.85
18*	Cipro 500	48.15	61.90	40.00

Table 6: Penetration of ciprofloxacin after 30 min based on percentages of the calibrated bulk fluid concentration and the low and high confidence interval limits of the bulk fluid concentration. * indicates erroneous data.

Exp.	Treatment ($\mu\text{g ml}^{-1}$)	% Calib. Bulk	% Low C.I.	% High C.I.
16	Cipro 100	89.47	170.00	56.67
19	Cipro 100	94.74	180.00	60.00
17	Cipro 250	153.85	200.00	131.58
20	Cipro 250	132.31	172.00	113.58
15	Cipro 500	77.78	100.00	64.62
18*	Cipro 500	42.59	54.76	35.38

Modeling of transport

The transport of levofloxacin and ciprofloxacin to uncolonized IREs was adequately simulated using a mathematical model based solely on the process of molecular diffusion (Figures 36 through 41). For a calculated diffusion coefficient, based on chemical structure, of the antibiotic in water ($4.0 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$), a liquid boundary layer thickness of $136 \mu\text{m}$ was estimated using AQUASIM.

The diffusion model with the parameters stated above was applied to transport data from the colonized IRE experiments. Figures 42 through 47 show that diffusion alone (simulation #1) was not able to model the antibiotic delivery curves. In simulation #2, the diffusion coefficient of the antibiotic was allowed to vary during parameter estimation. Again, the model was unable to capture prominent features of the experimental data.

When a model containing the processes of diffusion, adsorption, and desorption was applied to the experimental data, better fits were achieved (Figures 48 through 53). In this model the antibiotic diffusion coefficient, D_A , the rate constants for adsorption, K_S , and desorption, K_D , and the concentration of binding sites, S_0 , were allowed to vary during parameter estimation. Values for the diffusion coefficient, the ratio of the estimated diffusion coefficient to the calculated value ($D_{A\text{sim}}/D_{A\text{calc}}$), and the ratio of adsorption to desorption ($K_S \cdot S/K_D$) were estimated simultaneously using AQUASIM and are shown in Table 7. Although the model is a quantitative tool, the results it provided were used qualitatively, as order of magnitude estimates, and little significance was attached to exact parameter values.

The average diffusion coefficient for levofloxacin was $2.284 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, approximately 57% of the calculated value. The ratio of adsorption to desorption appeared to be dependent on the concentration of antibiotic. The average diffusion coefficient of ciprofloxacin was $1.539 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, approximately 38.5% of the calculated value. The adsorption-desorption ratio appeared fairly constant, unlike levofloxacin, with a unitless average value of 7.734.

Table 7: Parameter values obtained from fitting the experimental delivery data with a model incorporating the processes of diffusion, adsorption, and desorption. Using AQUASIM, parameter estimations were obtained for D_A , K_D , K_S , and S_0 . * indicates erroneous data that was not analyzed.**

Experiment	Treatment ($\mu\text{g ml}^{-1}$)	D_A ($\text{cm}^2 \text{s}^{-1}$)	$D_{A\text{sim}}/D_{A\text{calc}}$ (%)	K_S^*S/K_D
16	Levo 100	6.917 E-6	172.92	1680.665
19	Levo 100	4.983 E-7	12.46	149.430
17	Levo 250	1.575 E-6	39.38	5.114
20	Levo 250	***	***	***
15	Levo 500	1.323 E-6	33.08	0.317
18	Levo 500	1.105 E-6	27.63	0.850
	Average	2.284 E-6	57.10	367.27
16	Cipro 100	1.182 E-6	29.54	5.253
19	Cipro 100	4.433 E-6	110.83	13.719
17	Cipro 250	5.233 E-7	13.08	3.344
20	Cipro 250	1.145 E-6	28.63	8.087
15	Cipro 500	4.150 E-7	10.38	8.267
18	Cipro 500	***	***	***
	Average	1.539 E-6	38.49	7.734

Levofloxacin

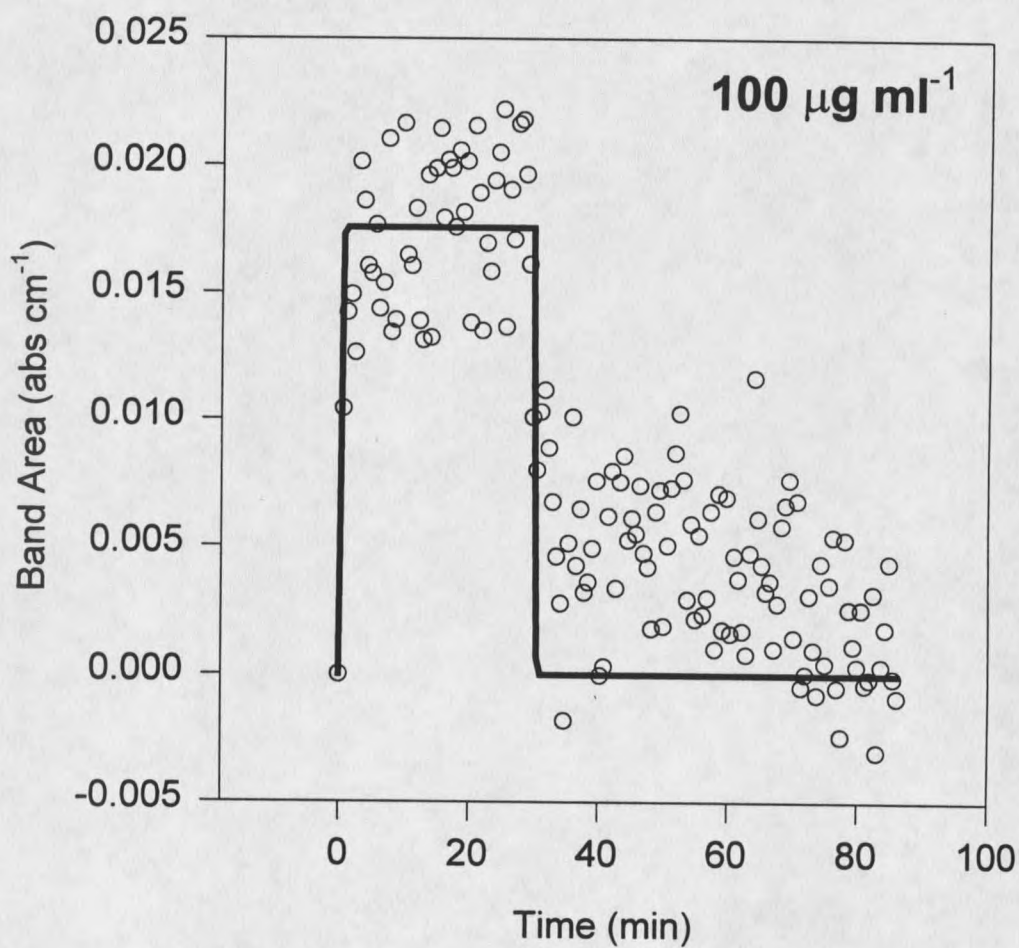


Figure 36: Model simulation (—) of levofloxacin at 100 µg ml⁻¹, experiment #22 (○), delivered to an uncolonized IRE. The simulation was run using AQUASIM and incorporated the process of diffusion.

Levofloxacin

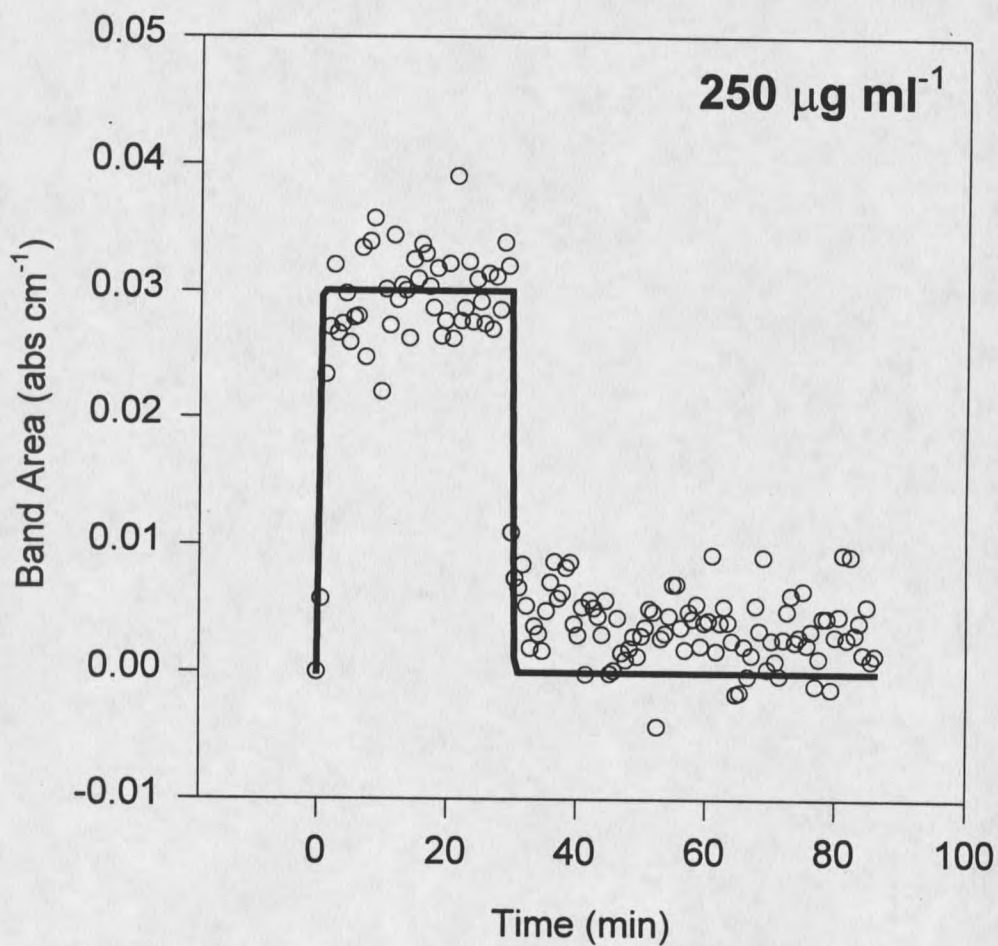


Figure 37: Model simulation (—) of levofloxacin at 250 µg ml⁻¹, experiment #23 (○), delivered to an uncolonized IRE. The simulation was run using AQUASIM and incorporated the process of diffusion.

Levofloxacin

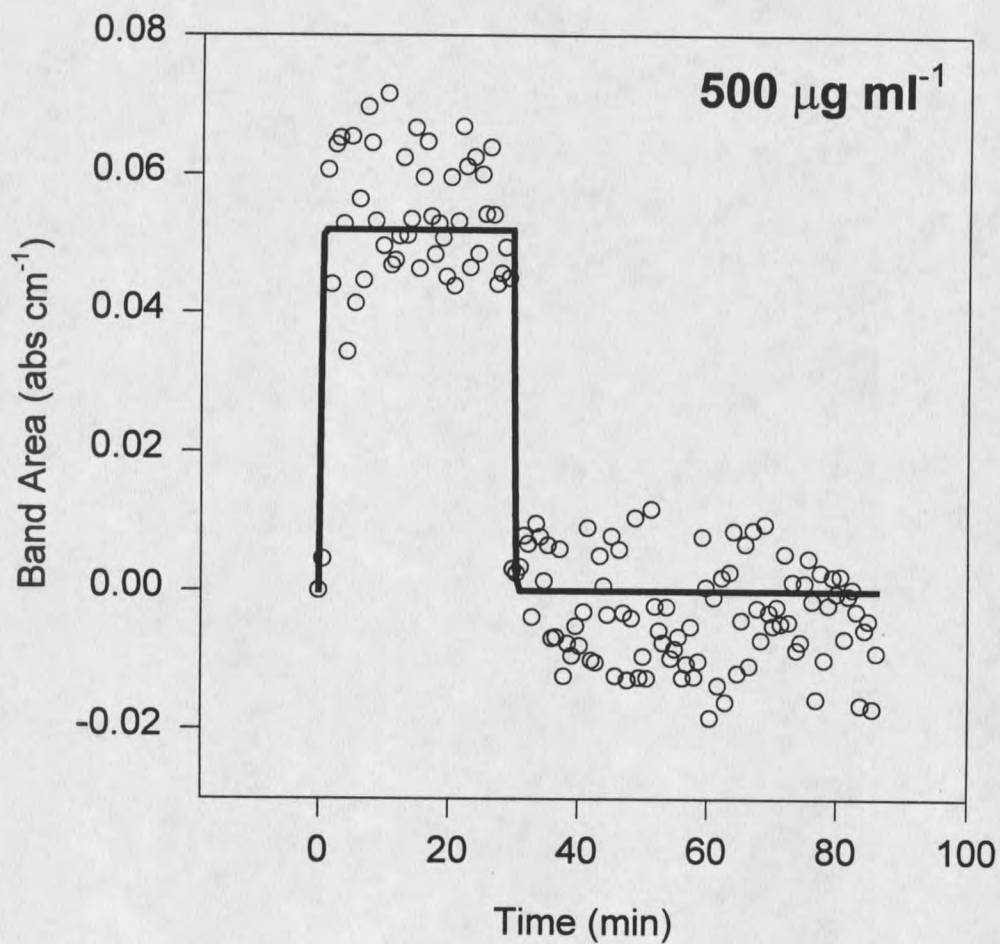


Figure 38: Model simulation (—) of levofloxacin at 500 µg ml⁻¹, experiment #27 (○), delivered to an uncolonized IRE. The simulation was run using AQUASIM and incorporated the process of diffusion.

Ciprofloxacin

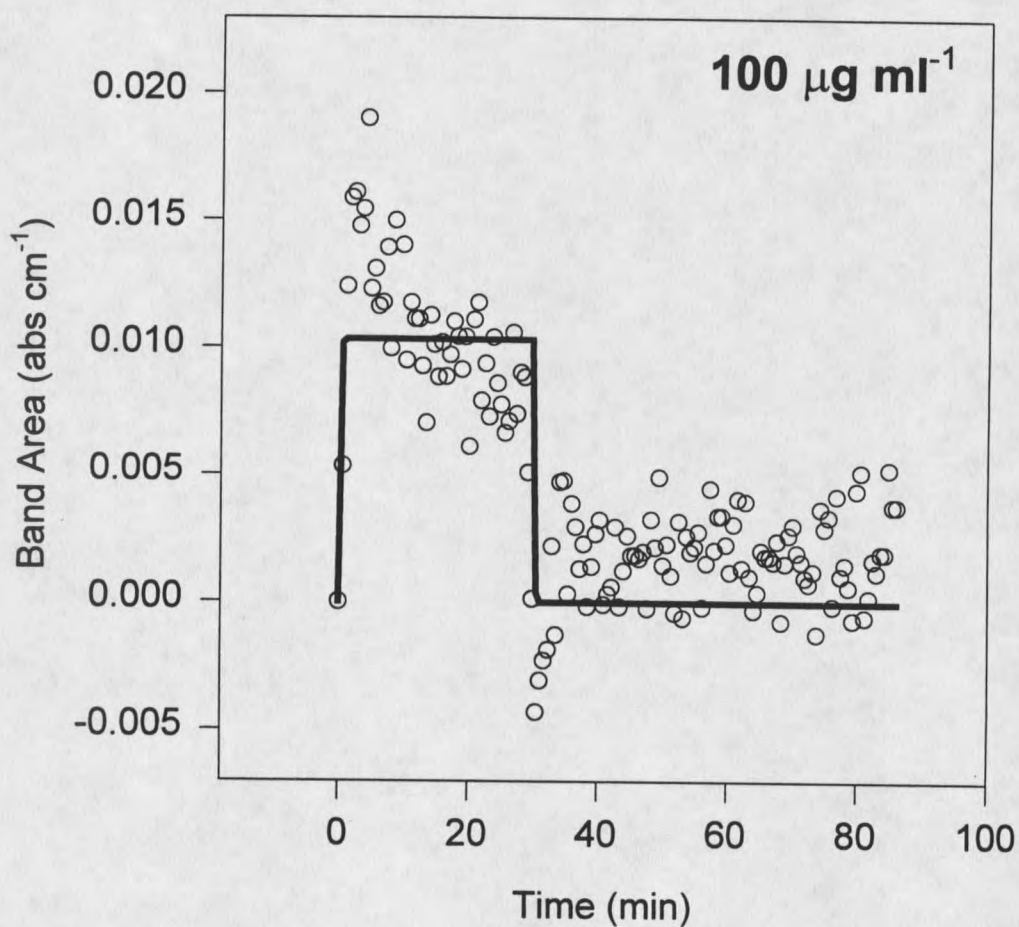


Figure 39: Model simulation (—) of ciprofloxacin at 100 μg ml⁻¹, experiment #22 (○), delivered to an uncolonized IRE. The simulation was run using AQUASIM and incorporated the process of diffusion.

Ciprofloxacin

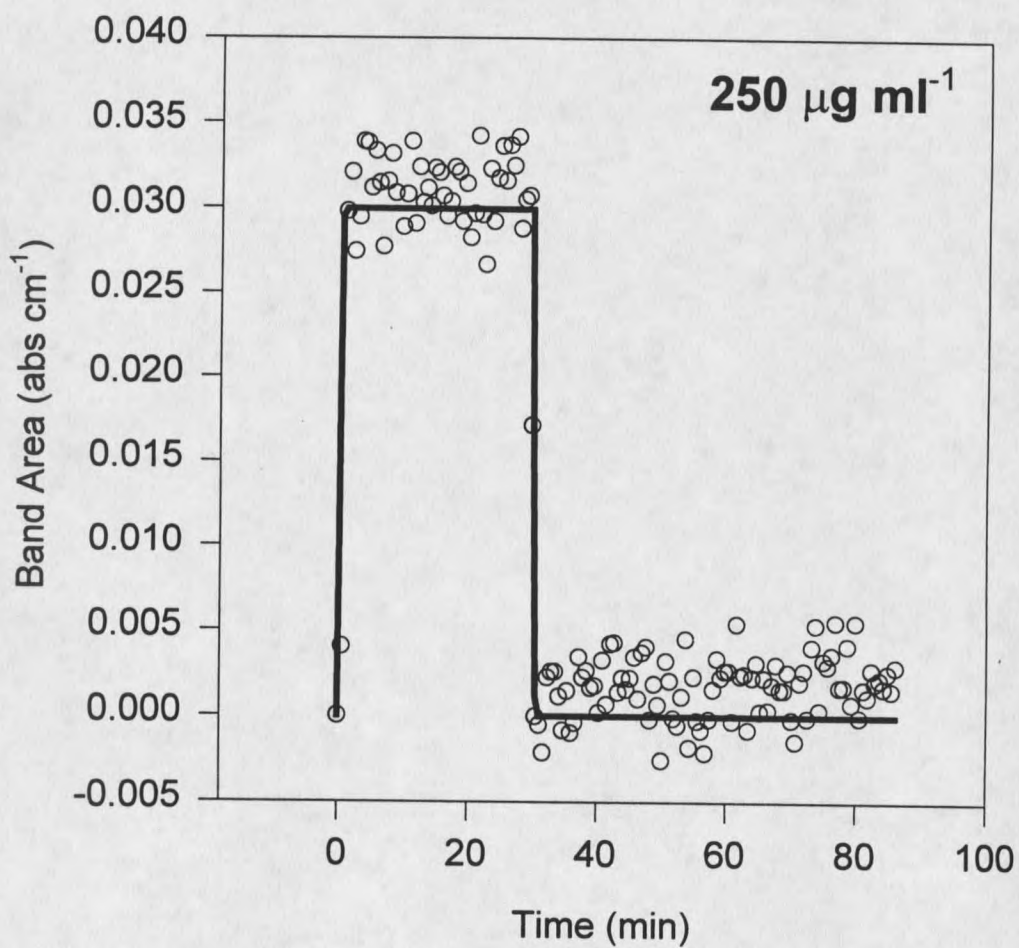


Figure 40: Model simulation (—) of ciprofloxacin at $250 \mu\text{g ml}^{-1}$, experiment #23 (○), delivered to an uncolonized IRE. The simulation was run using AQUASIM and incorporated the process of diffusion.

Ciprofloxacin

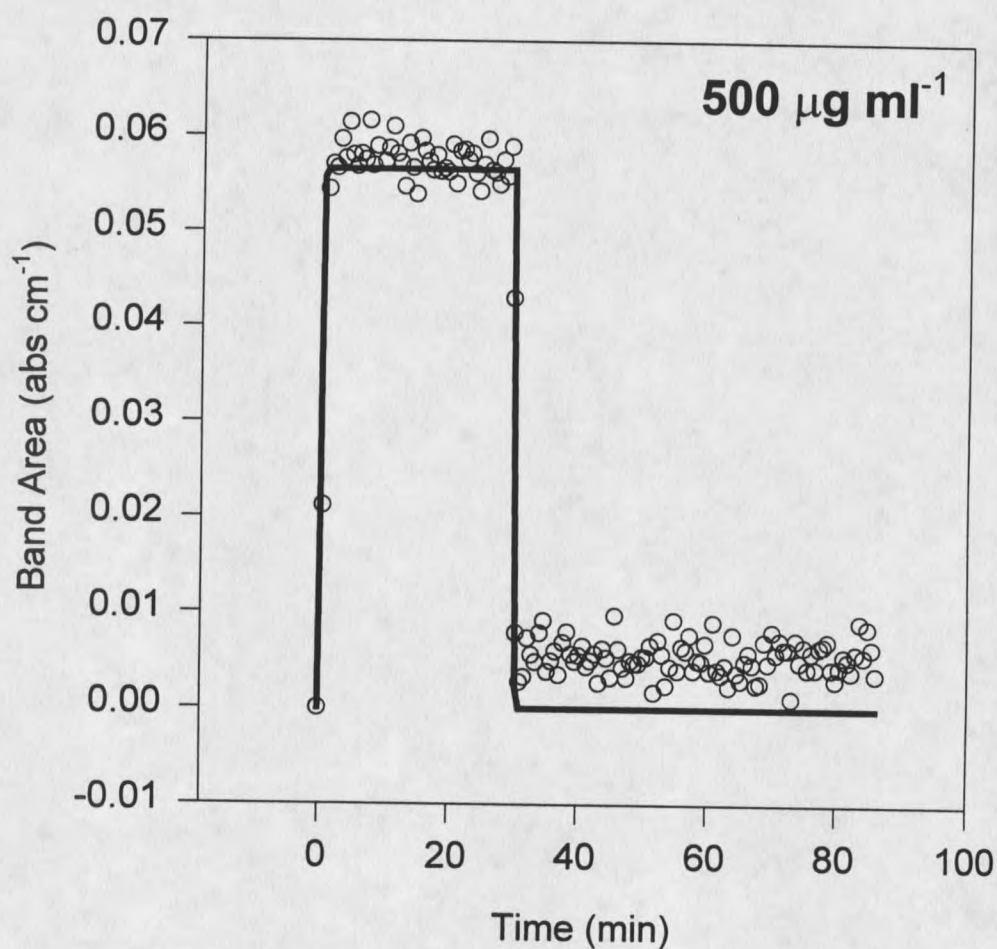


Figure 41: Model simulation (—) of ciprofloxacin at 500 μg ml⁻¹, experiment #27 (○), delivered to an uncolonized IRE. The simulation was run using AQUASIM and incorporated the process of diffusion.

Levofloxacin

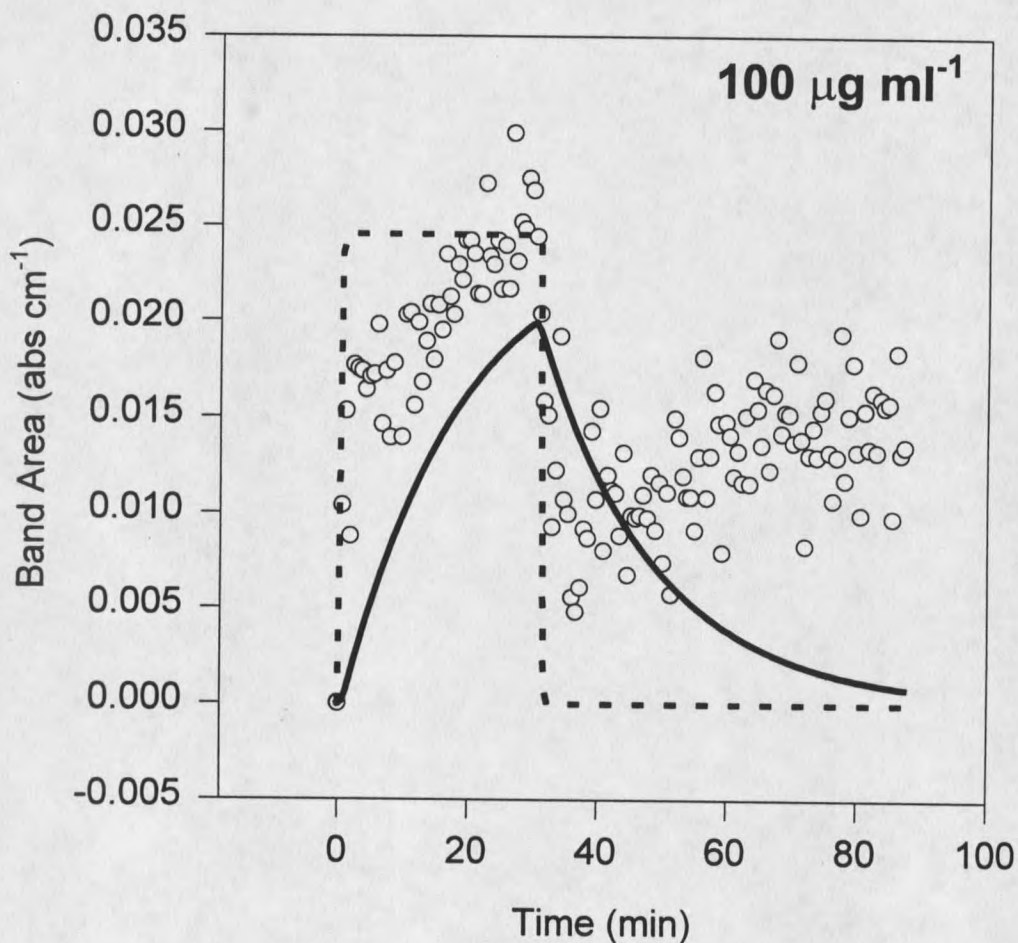


Figure 42: Model simulations of levofloxacin at $100 \mu\text{g ml}^{-1}$, experiment #19 ($\circ$), delivered to a colonized IRE. The simulations were run using AQUASIM and incorporated the process of diffusion. Simulation #1 (---) used the estimated value of the antibiotic diffusion coefficient in water. In simulation #2 (—) the value of D_A was allowed to vary to achieve the best fit.

Levofloxacin

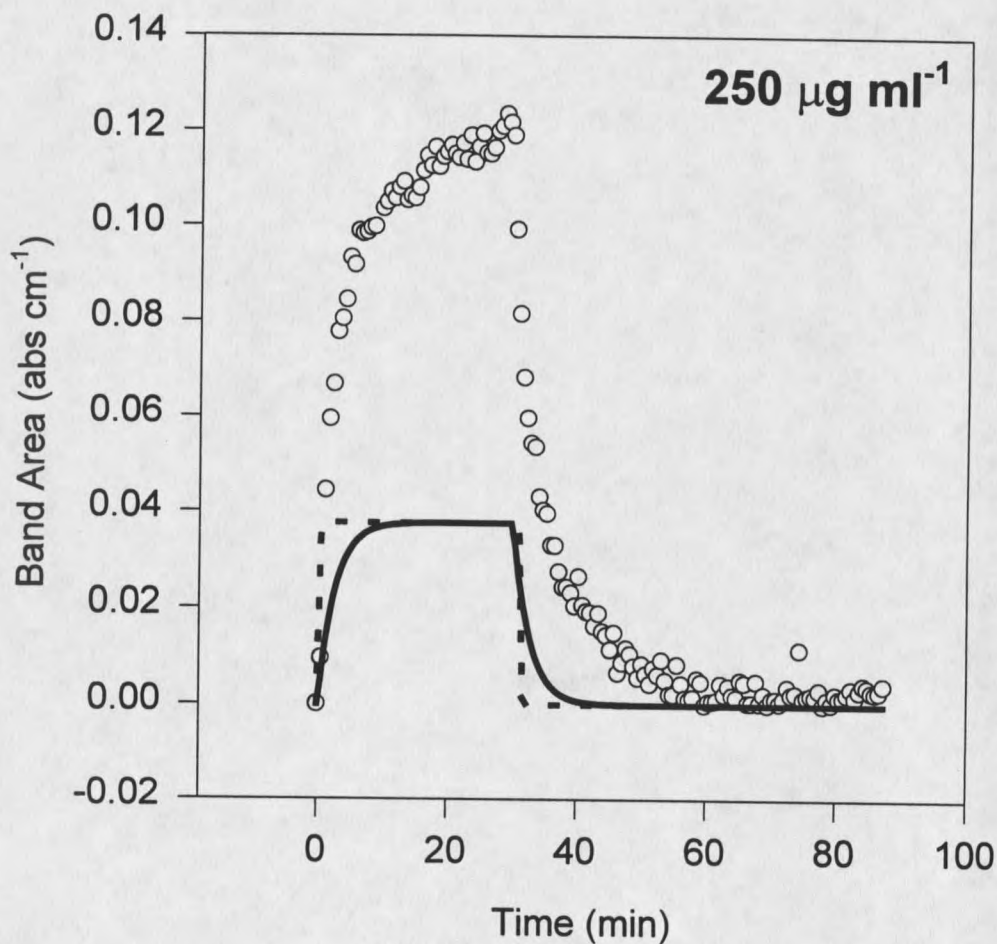


Figure 43: Model simulations of levofloxacin at 250 $\mu\text{g ml}^{-1}$, experiment #17 (○), delivered to a colonized IRE. The simulations were run using AQUASIM and incorporated the process of diffusion. Simulation #1 (---) used the estimated value of the antibiotic diffusion coefficient in water. In simulation #2 (—) the value of D_A was allowed to vary to achieve the best fit.

Levofloxacin

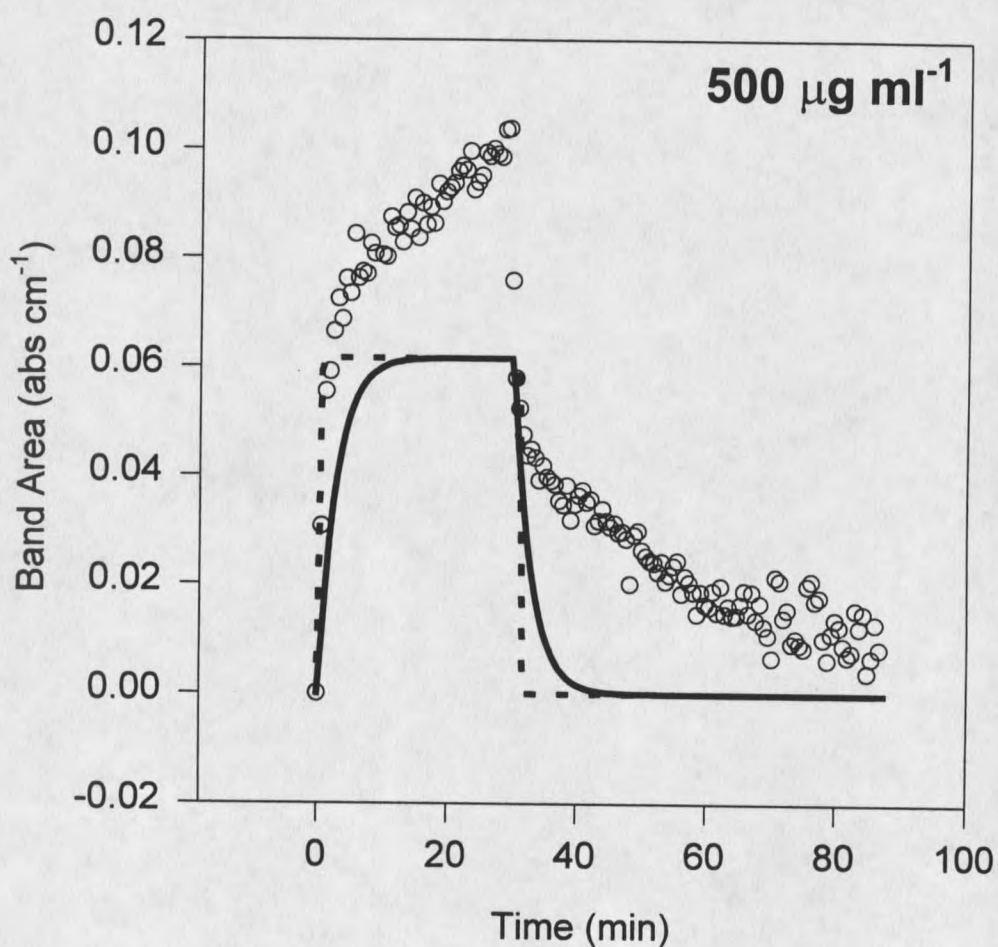


Figure 44: Model simulations of levofloxacin at 500 $\mu\text{g ml}^{-1}$, experiment #15 (○), delivered to a colonized IRE. The simulations were run using AQUASIM and incorporated the process of diffusion. Simulation #1 (---) used the estimated value of the antibiotic diffusion coefficient in water. In simulation #2 (—) the value of D_A was allowed to vary to achieve the best fit.

Ciprofloxacin

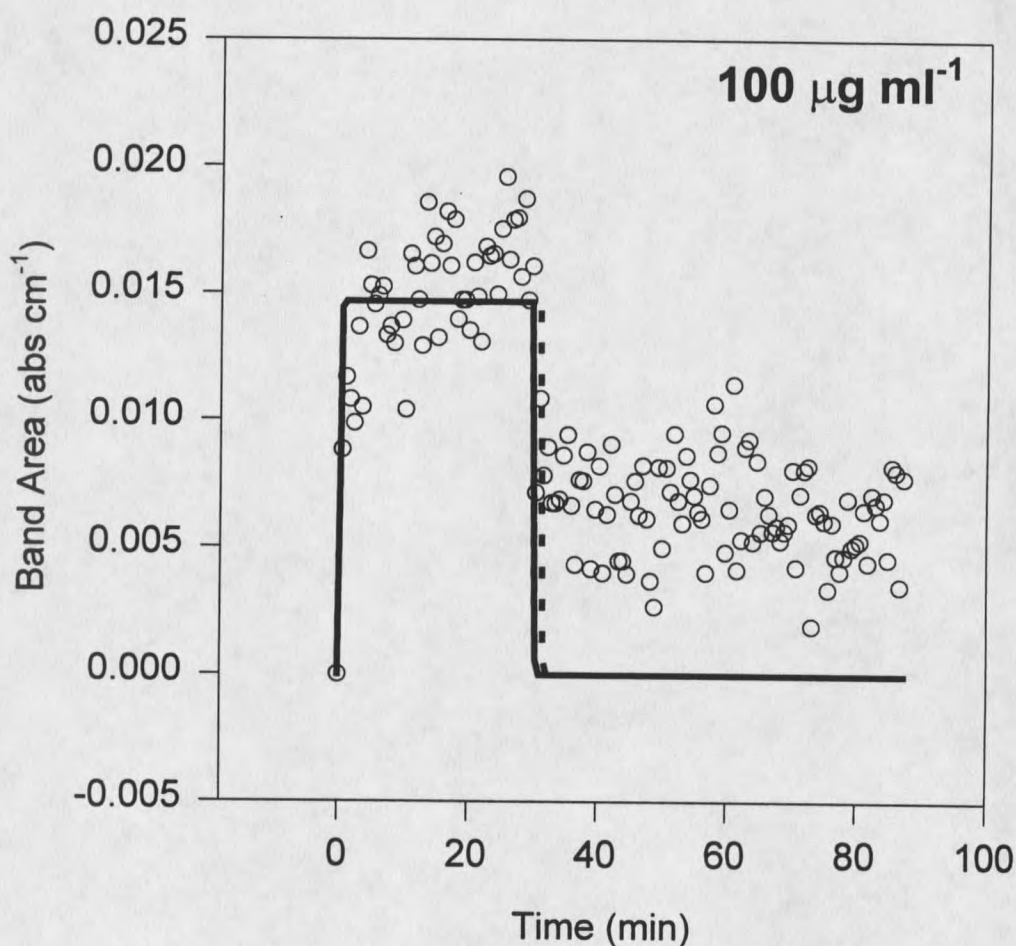


Figure 45: Model simulations of ciprofloxacin at 100 μg ml⁻¹, experiment #19 (○), delivered to a colonized IRE. The simulations were run using AQUASIM and incorporated the process of diffusion. Simulation #1 (---) used the estimated value of the antibiotic diffusion coefficient in water. In simulation #2 (—) the value of D_A was allowed to vary to achieve the best fit.

Ciprofloxacin

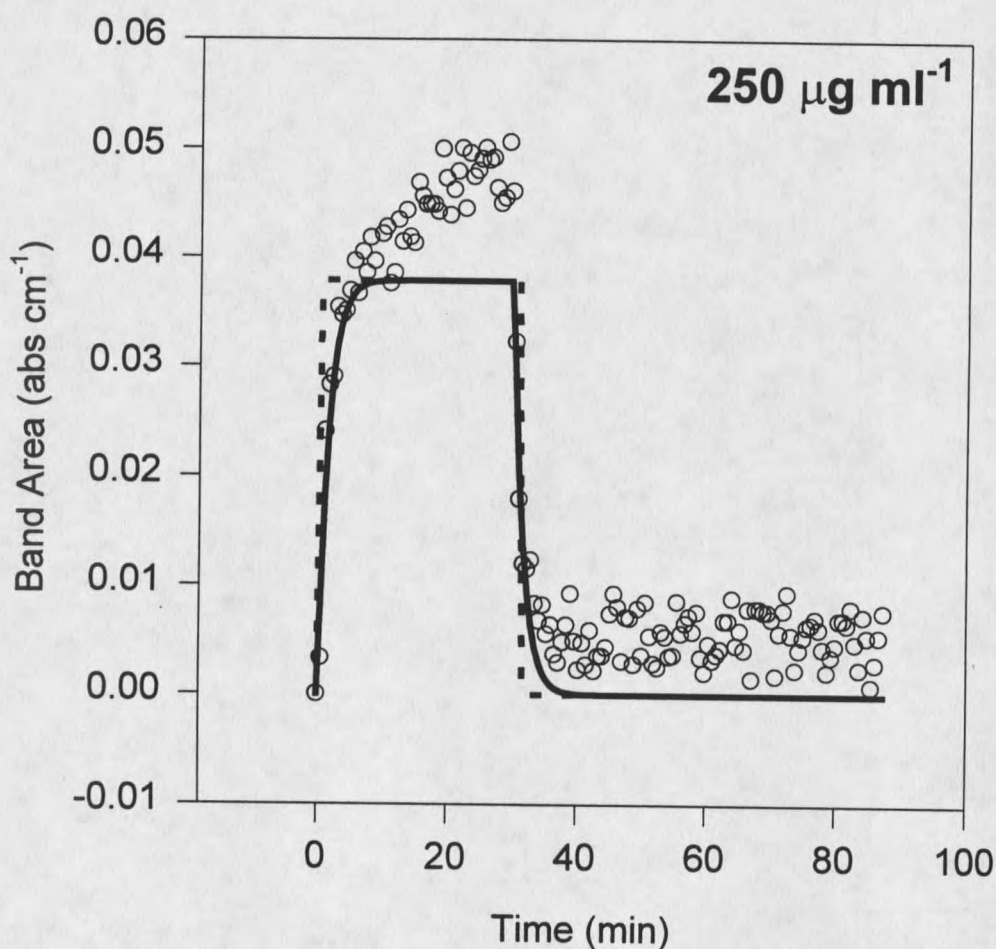


Figure 46: Model simulations of ciprofloxacin at 250 μg ml⁻¹, experiment #17 (○), delivered to a colonized IRE. The simulations were run using AQUASIM and incorporated the process of diffusion. Simulation #1 (- - -) used the estimated value of the antibiotic diffusion coefficient in water. In simulation #2 (—) the value of D_A was allowed to vary to achieve the best fit.

Ciprofloxacin

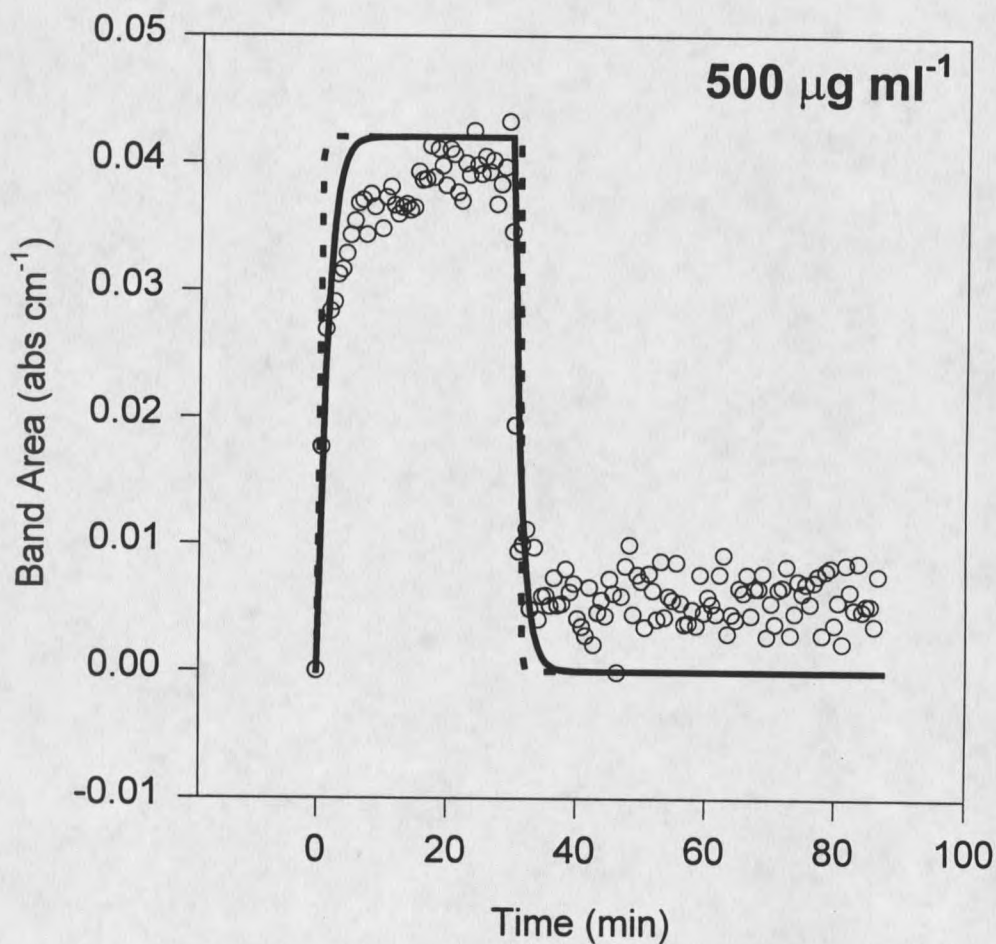


Figure 47: Model simulations of ciprofloxacin at $500 \mu\text{g ml}^{-1}$, experiment #15 ($\circ$), delivered to a colonized IRE. The simulations were run using AQUASIM and incorporated the process of diffusion. Simulation #1 (---) used the estimated value of the antibiotic diffusion coefficient in water. In simulation #2 (—) the value of D_A was allowed to vary to achieve the best fit.

Levofloxacin

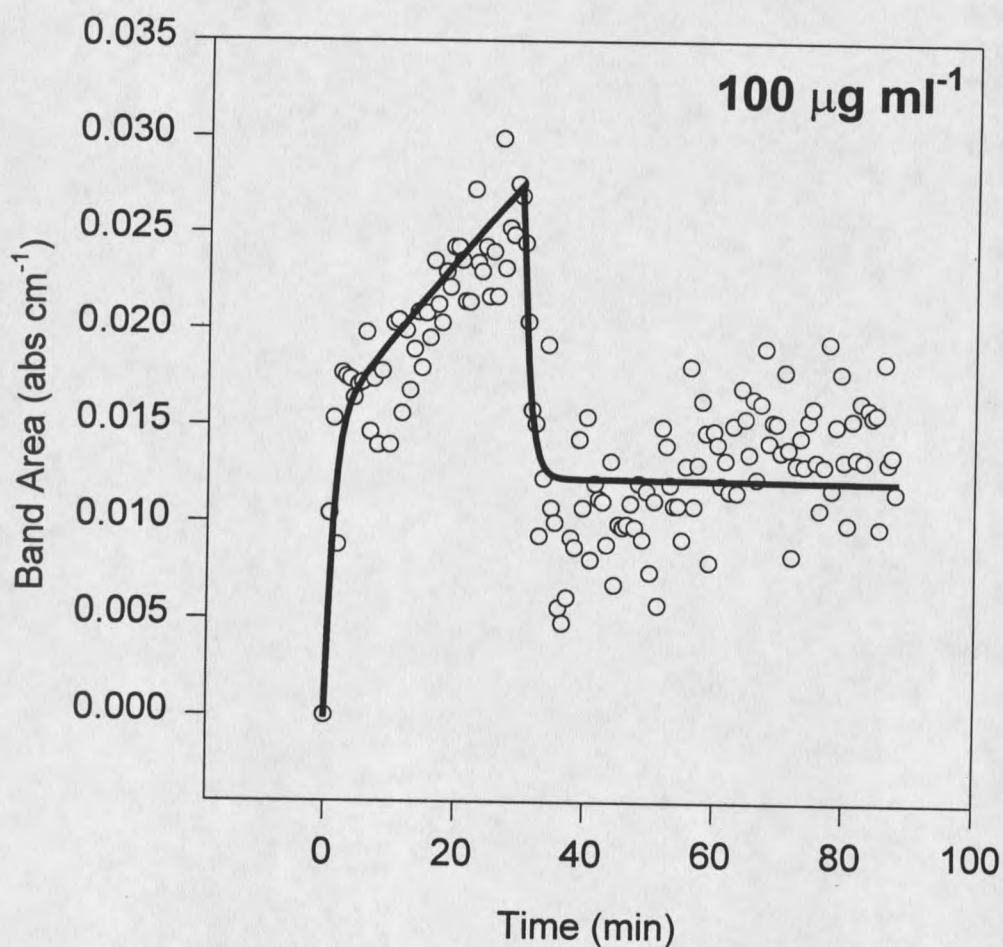


Figure 48: Model simulation (—) of levofloxacin at $100 \mu\text{g ml}^{-1}$, experiment #19 (○), delivered to a colonized IRE. The simulation incorporated the processes of diffusion, adsorption, and desorption, and was run using AQUASIM to estimate the parameters D_A , K_D , K_S , and S_0 .

Levofloxacin

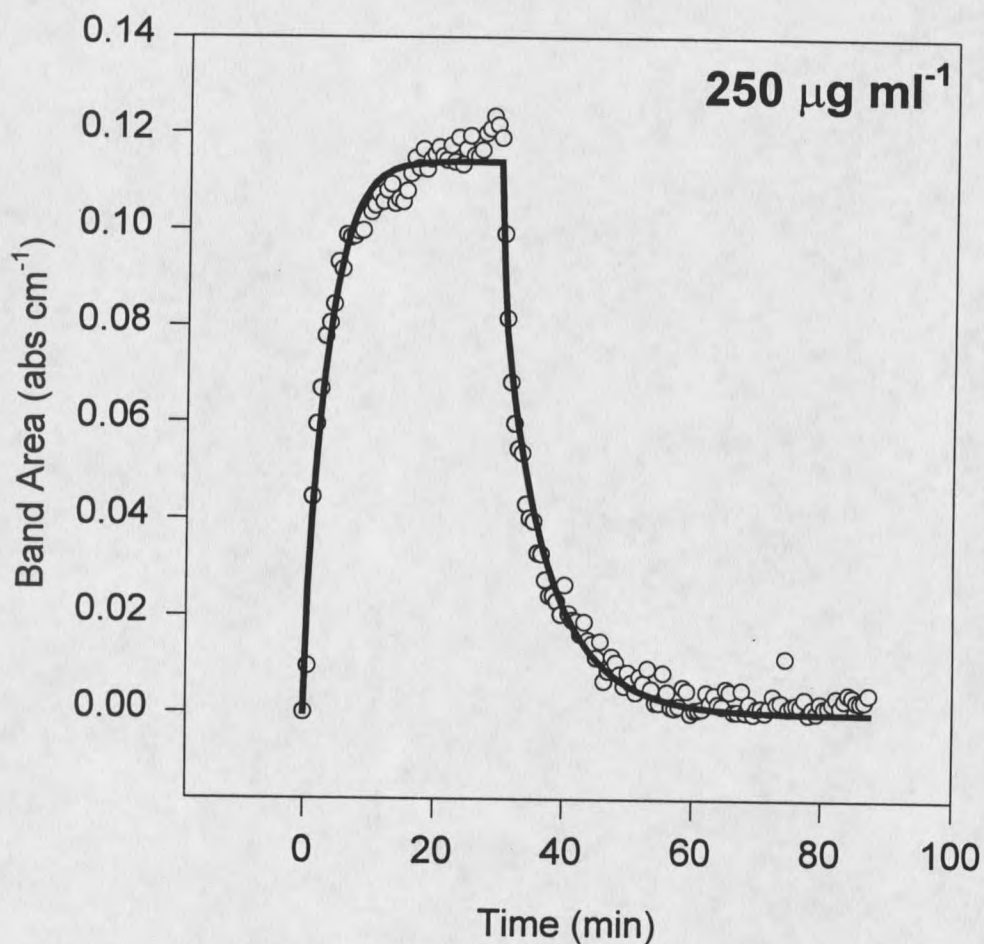


Figure 49: Model simulation (—) of levofloxacin at $250 \mu\text{g ml}^{-1}$, experiment #17 (○), delivered to a colonized IRE. The simulation incorporated the processes of diffusion, adsorption, and desorption, and was run using AQUASIM to estimate the parameters D_A , K_D , K_S , and S_0 .

Levofloxacin

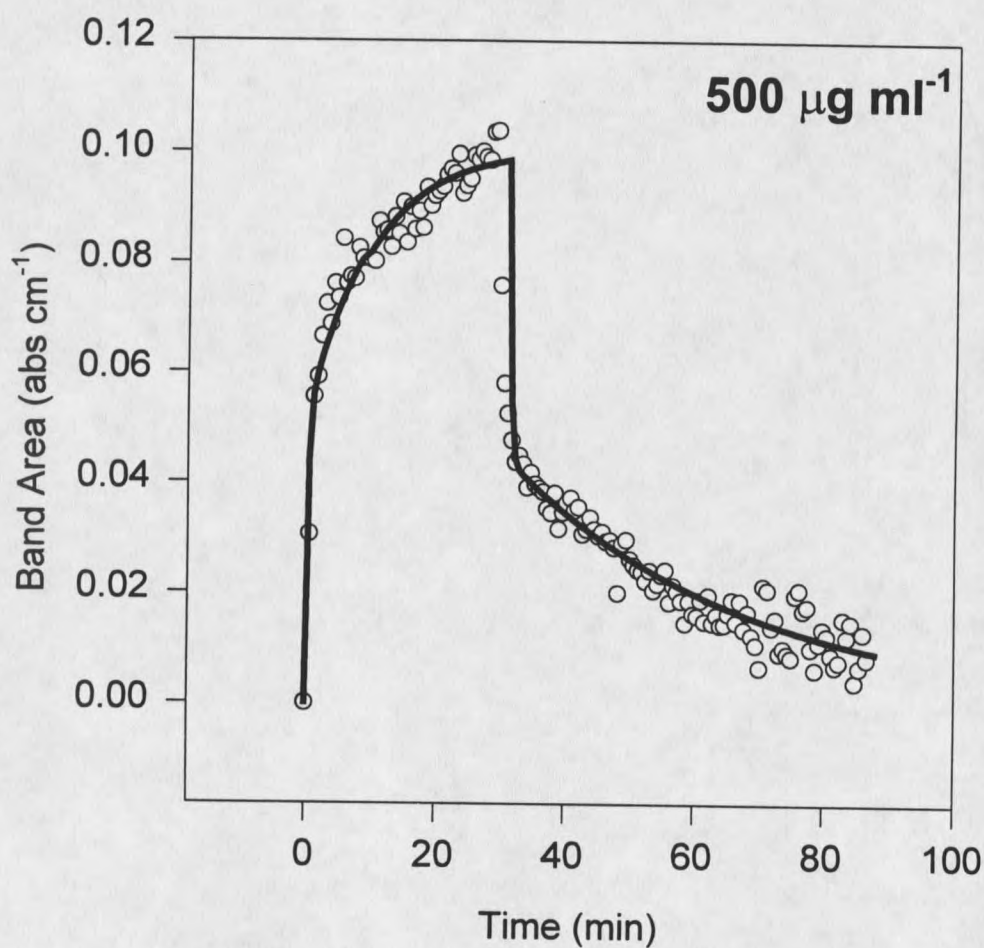


Figure 50: Model simulation (—) of levofloxacin at $500 \mu\text{g ml}^{-1}$, experiment #15 ($\circ$), delivered to a colonized IRE. The simulation incorporated the processes of diffusion, adsorption, and desorption, and was run using AQUASIM to estimate the parameters D_A , K_D , K_S , and S_o .

Ciprofloxacin

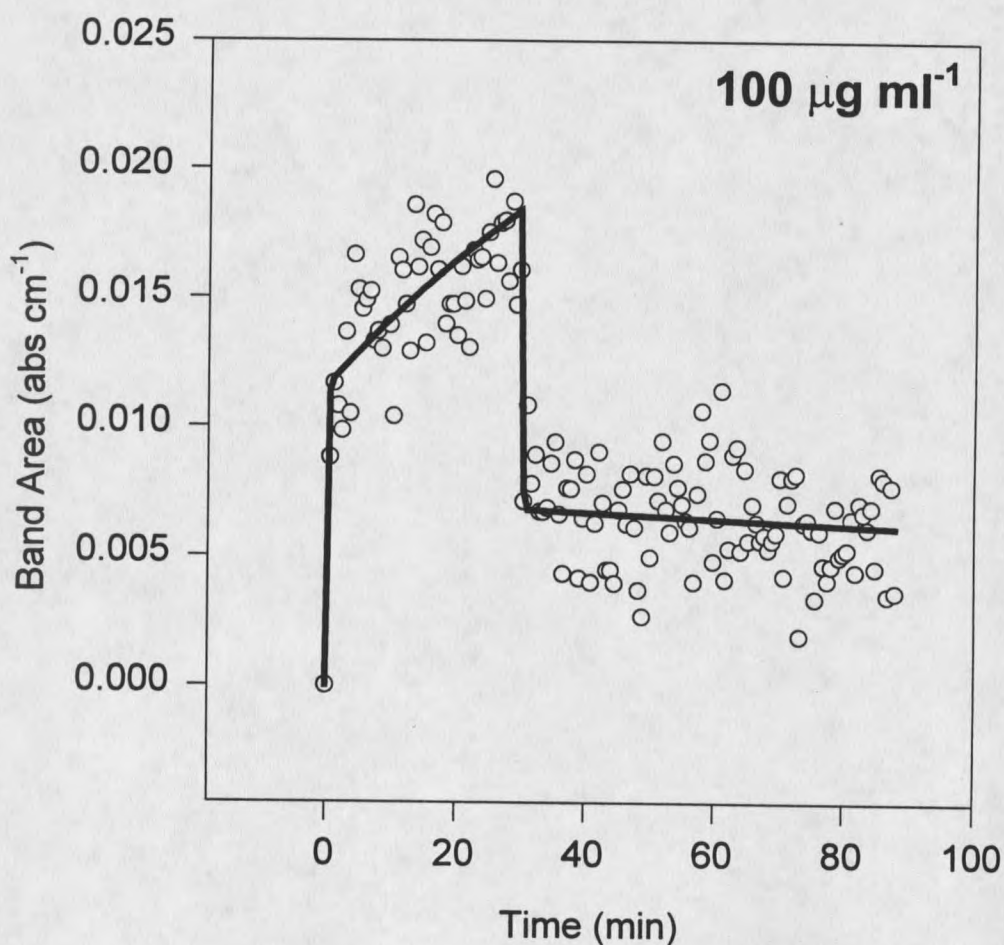


Figure 51: Model simulation (—) of ciprofloxacin at 100 µg ml⁻¹, experiment #19 (○), delivered to a colonized IRE. The simulation incorporated the processes of diffusion, adsorption, and desorption, and was run using AQUASIM to estimate the parameters D_A , K_D , K_S , and S_o .

Ciprofloxacin

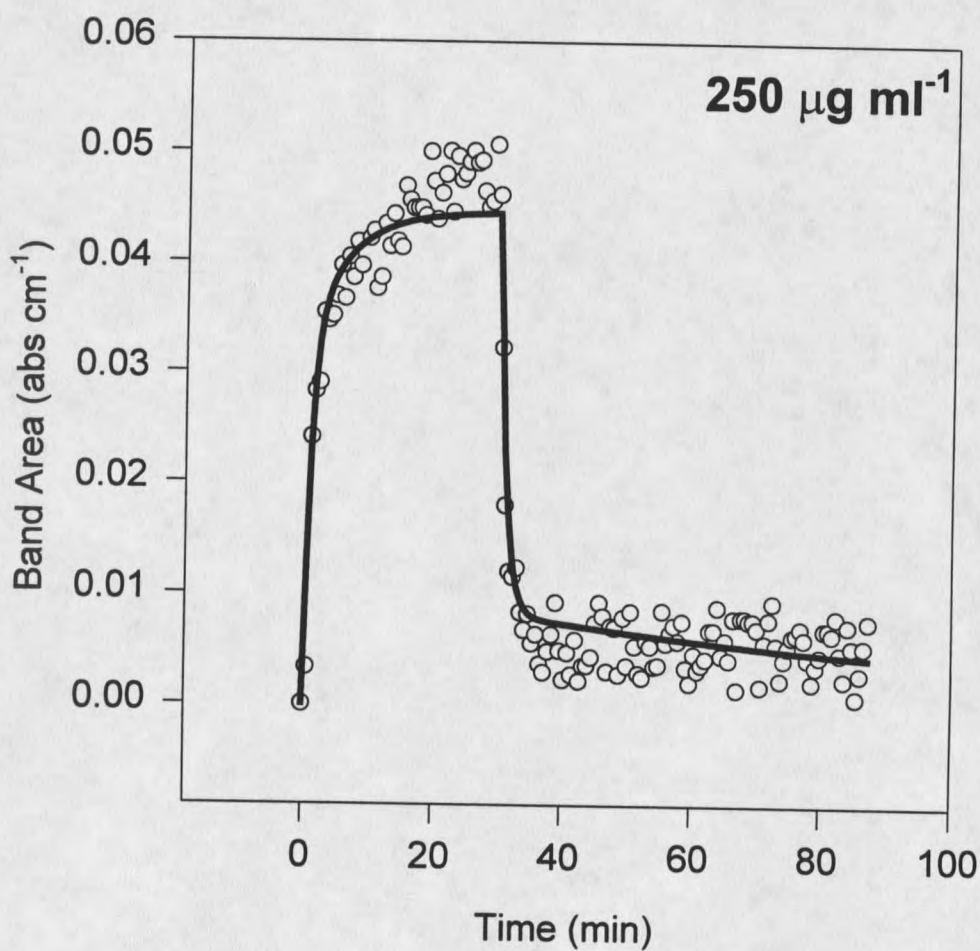


Figure 52: Model simulation (—) of ciprofloxacin at 250 $\mu\text{g ml}^{-1}$, experiment #17 (○), delivered to a colonized IRE. The simulation incorporated the processes of diffusion, adsorption, and desorption, and was run using AQUASIM to estimate the parameters D_A , K_D , K_S , and S_0 .

Ciprofloxacin

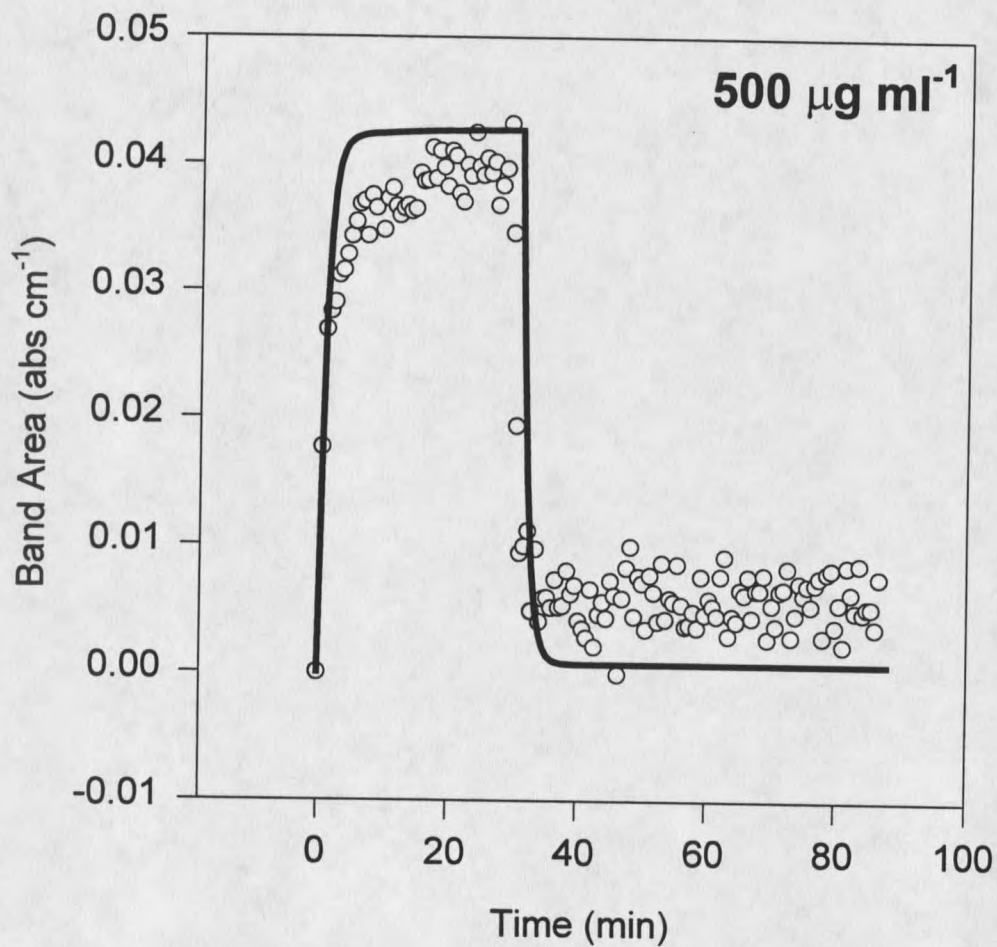


Figure 53: Model simulation (—) of ciprofloxacin at 500 µg ml⁻¹, experiment #15 (○), delivered to a colonized IRE. The simulation incorporated the processes of diffusion, adsorption, and desorption, and was run using AQUASIM to estimate the parameters D_A , K_D , K_S , and S_0 .

DISCUSSION

The goal of this study was to determine whether transport limitation of an antimicrobial agent through a biofilm contributed to antimicrobial resistance in biofilm bacteria. Our investigation found that the biofilm did not provide a penetration barrier to either ciprofloxacin or levofloxacin since bactericidal concentrations were attained at the substratum during the antimicrobial treatment. However, the presence of a biofilm did affect the accumulation of antibiotic at the biofilm-substratum interface. The results of this study imply that processes other than diffusion, particularly adsorption and desorption, could affect the concentration of antibiotics found in biofilms. Despite successful antibiotic penetration of the biofilm, biofilm populations were less susceptible to antibiotic treatments than planktonic cells. This suggests that physiologically based mechanisms could play a role in protecting biofilm cells from antimicrobial treatments.

The presence of a biofilm was not shown to significantly affect the rate of antibiotic transport to the biofilm-substratum interface. FTIR investigation of antibiotic penetration into biofilms revealed a rapid rise in concentration at the substratum of both levofloxacin and ciprofloxacin within the first 3 minutes of delivery, followed by a gradual increase over the remaining 30 minute treatment

(Figures 30 through 35). Within the first 5 minutes of antibiotic dosing, the concentration of levofloxacin at the substratum was found to be at least 79% of the calibrated bulk fluid concentration (with a 95% confidence interval range of 68 to 113%) while ciprofloxacin reached at least 65% (with a 95% confidence interval range of 54 to 83%) (Tables 3 and 5). At 30 minutes, levofloxacin had either attained or exceeded the bulk fluid concentration based on the concentration-band area calibration curve and its confidence intervals (Table 4) while the substratum concentration of ciprofloxacin was at least 78% of the bulk fluid concentration (95% confidence interval range of 65 to 100%) (Table 6). Because of the scatter in the uncolonized IRE experimental data (Figures 20 through 25), associated with the experimental technique, it is difficult to ascertain the direct effect of the biofilm on antibiotic transport rates. However, it is clear from these results that the transport barrier presented by the 15-20 μm thick biofilm of *Pseudomonas aeruginosa* was not large enough to prevent bactericidal antibiotic concentrations from reaching the biofilm-substratum interface.

In order to accurately monitor the antibiotic penetration through a biofilm using the ATR-FTIR technique, it was necessary to obtain a uniform biofilm that completely covered the IRE surface. The biofilm architecture was important in interpreting whether the administered antibiotic was contacting the IRE surface by diffusing through the biofilm or whether it was reaching the substratum only in

uncolonized areas. The architecture of the biofilms in this study was shown to be "loosely knit" in regions (Figures 15 and 16), but the substratum was found to be completely colonized by at least 2-3 cell layers (Figure 11) and all biofilms averaged between 15 and 20 μm thick (Figures 13 through 18). Although the matrix exopolysaccharide (EPS) architecture and coverage was not examined in this study, other investigations of biofilm structure have determined that an EPS matrix extends between loosely knit cell clusters (Stewart et al., 1995).

Consequently, there is reason to believe that EPS connected the loosely knit cell clusters seen in the cryosections. If there had been bare areas devoid of cells and EPS matrix on the IRE and the antibiotic only reached the substratum through these uncolonized areas, the antibiotic FTIR spectra from the colonized IRE experiments should have had lower absorbance values than the FTIR spectra obtained from the uncolonized experiments. These lower absorbance values would have resulted in smaller band area values for the antibiotic delivery curves. However, this was not the case. Even if the antibiotic did not penetrate the biofilm patches, but sorbed to the biomass adjacent to void areas, the FTIR signal should have been less than the signal from the uncolonized IRE. In our experiments, the band area values of colonized IRE experiments approached, if not exceeded, levels established when there was no biofilm. This suggests that the antibiotic was fully penetrating the biofilm and that no diffusion barrier was present.

Although the presence of a biofilm did not significantly hinder the transport of antibiotic to the substratum, it did affect the shape of the delivery curve and the concentration of antibiotic at the substratum during treatment. Antibiotic delivery curves from experiments containing biofilms were shaped differently than the uncolonized IRE experimental curves. Unlike the uncolonized IRE delivery curves, which plateaued after 3 minutes and maintained a steady state concentration, the substratum concentration continued to gradually rise, often above the bulk fluid concentration, throughout an experiment when a biofilm was present. This FTIR data suggests that diffusive transport of both fluoroquinolone antibiotics into a biofilm was modified by sorption to biofilm components. The process of sorption could include binding of the antibiotic to cells, EPS, and biomass. The concept of antibiotic transport being altered by adsorption is not new. Nichols et al. (1988) showed that the transport of tobramycin through alginate was altered by binding and proposed a mathematical model to further explain this concept (Nichols et al., 1989).

To understand the extent of the sorption processes which may have occurred in this study, experimental antibiotic delivery curves were simulated using a mathematical model which was tailored to the experimental conditions and incorporated the transport processes of diffusion, adsorption, and desorption. Although the model is a quantitative tool, it was used in this study to provide qualitative insight into relevant transport processes. From the model, it

was ascertained that the process of diffusion could explain the rapid penetration of the antibiotic, but not the subsequent gradual increase in concentration during the remaining time of the antibiotic dose. A model comprising diffusion alone was not capable of simulating the rise of the concentration at the substratum above the bulk fluid concentration or the gradual antibiotic washout characteristics. Incorporating adsorption and desorption processes into the model allowed better simulation of the observed trends. During the modeling simulations the rates of adsorption and desorption, the concentration of binding sites, and the diffusion coefficient of the antibiotic were allowed to vary to achieve the best fits to the experimental data. The values of these parameters, as estimated by the model, were then compared.

From the model simulations, the ratio of adsorption to desorption rates was found to be fairly constant for ciprofloxacin while it varied over orders of magnitude for levofloxacin as a function of the bulk fluid concentration. The variability of the levofloxacin ratios suggests that the inherent model assumption of first order adsorption-desorption kinetics was incorrect for this agent. The between experiment variability of all estimated parameters implies that the parameters are not easily identified. It is possible that multiple solutions provide good fits to the experimental data, that the model assumptions were not completely correct, or that another process should be accounted for in the model. However, fits that accurately simulated the experimental data were

obtained using the first order adsorption-desorption kinetic parameters. This agreement implies that the processes of adsorption and desorption are relevant and that these processes provide a potential explanation for the experimental data trends. No relationship was found between the value of the adsorption-desorption ratio and antibiotic efficacy, but it is possible that the binding of an antibiotic to biofilm components could prevent bactericidal antibiotic concentrations from entering the cell.

Observations that biofilm bacteria are invariably more resistant to antimicrobial treatment than their planktonic counterparts were confirmed by the 30 minute exposure of planktonic and biofilm populations to levofloxacin. During this treatment time, levofloxacin produced a 4-fold greater killing of planktonic bacteria than biofilm populations (Figure 19). However, this reduced susceptibility of biofilm bacteria was not observed with ciprofloxacin, a structural analog of levofloxacin, challenging the dogma that currently exists in the field. One explanation for the lack of a population-dependent resistance response may be a reduced susceptibility of both planktonic and biofilm bacteria to ciprofloxacin compared to planktonic bacteria treated with levofloxacin. The decreased efficacy of ciprofloxacin could also be related to exposure time. Another possible interpretation of decreased ciprofloxacin efficacy in the planktonic and biofilm populations is a "paradoxical response" of both bacterial populations to the high concentrations of ciprofloxacin. In the paradoxical

response, treatment with excessive concentrations of antibiotic can be counterproductive. Bacteria exposed to an antibiotic at concentrations exceeding 100 times the minimum inhibitory concentration (MIC) may be less susceptible to the antimicrobial agent than bacteria treated with concentrations closer to the MIC (Holm et al., 1991). Because bacteria have different susceptibilities to different antibiotics, it is possible for a paradoxical response to have occurred for one antibiotic and not the other, even when the antibiotics are similar in chemical structure.

The similar susceptibilities of the biofilm and planktonic populations to ciprofloxacin is consistent with the observation of rapid ciprofloxacin penetration into the biofilm and lack of significant transport limitation. The near-equivalent efficacies suggest that the rate of ciprofloxacin transport within the biofilm is not a factor in biofilm recalcitrance to this agent. Since there was no significant transport limitation within the biofilm and both biofilm and planktonic cells were exposed to the same antibiotic concentrations, the efficacy of the antibiotic against both types of organisms would be expected to be equivalent unless the biofilm cells were physiologically different from the planktonic organisms and inherently resistant.

In the biofilm case of treatment with levofloxacin, which demonstrated a clear reduction in antibiotic efficacy compared to a planktonic control, a resistance mechanism other than transport limitation of the agent is indicated.

One possible explanation for non-transport related resistance is a difference between the cell physiologies of attached, biofilm bacteria and free, planktonic organisms. Brown et al. (1988) suggests that biofilm cells differ from planktonic cultures in that they often grow more slowly. They have also shown that growth rate and nutrient limitation can affect antibiotic efficacy by altering cell physiology.

A second explanation for the resistance of biofilm populations exposed to levofloxacin could be a physiological response induced by the presence of the antibiotic. Although the antibiotic penetration curves show no significant transport limitations, modeling efforts have indicated that best fits to the experimental data were achieved with effective antibiotic diffusion coefficient values of approximately 38 to 57% of the calculated diffusion coefficient for the antibiotic in water. These values, which vary over one order of magnitude only, are consistent with literature reports that cite effective diffusion coefficients for transport within biofilms (Fan et al., 1989; Libicki et al., 1987) and eliminate the possibility that the biofilm acts as a huge barrier to diffusion. The estimated diffusion coefficient range of 1.5×10^{-6} to $2.3 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ indicates a delay in the appearance of the antibiotic at the substratum on the time scale of minutes. Although this delay is not significant when compared to the time scale of the overall dose, it is possible that this delay of approximately two to three minutes could allow enough time for bacteria to respond to the antibiotic invasion and

adapt to the hostile environment by activating resistance genes. If biofilm bacteria physiologically differ from planktonic organisms in their genetic expression and global regulation mechanisms, a resistance response to antimicrobials may be quicker and more effective in biofilm bacteria. An example of a resistance response initiated by the presence of an antibiotic is the production of β -lactamase enzymes which degrade β -lactam antibiotics (Giwerzman et al., 1991). Another example of a rapid survival response is the bacterial response to heat shock in *E. coli* where the degradation of a sigma factor protein is inhibited. This allows the protein to accumulate and offer resistance to the cell within a few minutes (Brock et al., 1994).

As shown by the transport curves, bactericidal concentrations of antibiotic surrounded the bacteria at the biofilm substratum. Modeling has indicated that the combination of diffusion, adsorption, and desorption processes could reproduce the experimental data trends of an antibiotic penetrating a biofilm. Thus, it is possible that adsorption-desorption processes were active in the biofilms studied and that these processes were responsible for the substratum concentration exceeding that of the bulk fluid. Because the biofilm bacteria were exposed to concentrations equivalent to or greater than that of the bulk fluid, the idea of the biofilm acting as a barrier to antimicrobial penetration does not apply to our study and other explanations of resistance, such as physiological mechanisms, must be explored.

CONCLUSIONS

This research involved treating *Pseudomonas aeruginosa* biofilms and planktonic cultures with the antibiotics ciprofloxacin and levofloxacin for 30 minutes. The treatment was followed by a 60 minute fresh media rinse and antibiotic efficacies were determined by quantifying viable and total cells. Antibiotic transport within biofilms was investigated by monitoring the appearance of the antibiotic at the biofilm-substratum interface with ATR-FTIR techniques. Within the limitations and scope of this study, the results have led to the following conclusions:

- 1) Biofilm populations were much less susceptible to levofloxacin than planktonic populations. Consequently, antibiotic efficacy tests should not be carried out using suspended cell cultures only.
- 2) There was little difference in susceptibility between planktonic and biofilm populations exposed to ciprofloxacin.
- 3) There was little difference between the susceptibilities of biofilm populations treated with either levofloxacin or ciprofloxacin.
- 4) Antibiotic delivery curves determined from colonized IRE experiments had different shapes than the curves from uncolonized IRE experiments.

- 5) Bactericidal concentrations of levofloxacin and ciprofloxacin were monitored via ATR-FTIR at the biofilm-IRE substratum interface.
- 6) A mathematical model containing only the process of diffusion was not capable of simulating the experimental antibiotic penetration trends when a biofilm was present.
- 7) A mathematical model containing the processes of adsorption, desorption, and diffusion adequately fit the experimental antibiotic penetration data for colonized IRE experiments.

RECOMMENDATIONS FOR FUTURE WORK

Further experimental work could support and strengthen the results obtained in this study. The transport conclusions presented earlier are specific to the structure of a 50 hour *Pseudomonas aeruginosa* biofilm and treatment for 30 minutes with the antibiotics ciprofloxacin and levofloxacin. To gain more insight about the role of transport limitation in the resistance of biofilm organisms to antimicrobials, three approaches could be taken based on this study. The first approach involves modifying the antibiotic treatment, the second focuses on changing the biofilm characteristics, and the third requires changes to make the experiments more medically relevant.

The transport and efficacy of other antibiotics and structural analog antimicrobials through biofilms could be explored. ATR-FTIR techniques could be used to monitor their transport in hopes of determining how transport is affected by chemical structure. An understanding of how much antibiotic is consumed by the cells, or bound by cell components and EPS, could be gained by correlating FTIR readings to an independent measure of the antibiotic such as radioactive labeling, thin layer chromatography, or fluorescence.

The second approach to expanding this study requires altering biofilm characteristics while ensuring that the biofilm is reproducible from experiment to

experiment. Biofilms of varying thicknesses, densities, and ages could be studied in terms of their permeabilities and interactions with antibiotics. In addition, monitoring the transport of antibiotics through dead, "inert", biofilms could provide insight to the binding processes of adsorption and desorption.

Now that a systematic experimental method has been developed, the medical relevance of the study could be improved. It would be ideal to lessen the antibiotic concentrations to a range that was medically relevant (to study resistance), but with the current sensitivity limitations of the FTIR instrumentation this is not possible. The biofilms in this study were grown directly on the germanium IRE so that transport of the antibiotics to the substratum could be easily monitored. However, germanium is not a medically relevant substratum. Because studies have shown that substratum chemistry and topography can affect biofilm formation, it would be advantageous to coat the IRE with a thin polymer film of a medically relevant material before initiating biofilm growth. If the polymer film was less than 0.5 μm thick, the ATR-FTIR technique could still be used to monitor antibiotic penetration. Finally, single species biofilms of *P. aeruginosa* were examined in this study, but the literature supports much evidence that medical devices are colonized by multiple organisms which form more complex, mixed-culture biofilms. The present study could be repeated to examine antibiotic transport through biofilms composed of other popular organisms such as *Staphylococcus aureus* and *Staphylococcus epidermidis*,

independently and in combination with each other. The most medically relevant study would grow a biofilm based on a mixed-culture inoculum, isolated from a colonized implant, and use a nutrient broth most similar to that supplied by the body.

My final recommendation for future work is to utilize a flat, trapezoidal IRE-sample chamber set-up so that the biofilm can be monitored periodically during the experiment with confocal microscopy, as well as with ATR-FTIR techniques. This would help establish a relationship between the structure of the biofilm and its transport characteristics.

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APPENDICES

APPENDIX A

AQUASIM Parameters**Table 8: Parameter values used for model simulation of experiments.**

Parameter	Symbol	Value	Units
Flow rate	Q	3.200 E-6	m ³ min ⁻¹
Reactor volume	V	2.890 E-7	m ³
Biofilm surface area	Area _{bf}	6.796 E-4	m ²
Biomass volume fraction	eps _x	0.0975	----
Intrinsic cell density	rho _x	4.700 E+4	g m ⁻³
Biofilm thickness	L _f	20.00 E-6	m
Liquid boundary layer thickness	L _l	136.0 E-6	m
Biofilm-bulk fluid diffusivity ratio	R	0.9000	----
Antibiotic diffusivity in water	D _A	2.400 E-8 or from fit	m ² s ⁻¹
Antibiotic concentration (free)	A	100, 250, or 500	g m ⁻³
Antibiotic concentration (bound)	A _s	from fit	g m ⁻³
Antibiotic dose duration	t _A	30	min
Yield coefficient for adsorption	Y _{SA}	1	----
Sorbing particulate concentration	S _o	from fit	g m ⁻³
Adsorption rate coefficient	K _s	from fit	m ³ g ⁻¹ min ⁻¹
Desorption rate coefficient	K _D	from fit	min ⁻¹

APPENDIX B

Viable and Total Cell Data**Table 9: Viable and total cell mean counts for planktonic cultures exposed to levofloxacin and ciprofloxacin.**

Exp.	Conc. ($\mu\text{g ml}^{-1}$)	Levofloxacin		Ciprofloxacin	
		Viable Cells (cfu ml^{-1})	Total Cells (cells ml^{-1})	Viable Cells (cfu ml^{-1})	Total Cells (cells ml^{-1})
1	100	4.00 E+3	4.99 E+7	3.17 E+5	3.29 E+7
2	100	1.57 E+3	3.29 E+7	1.13 E+6	2.98 E+7
3	100	1.12 E+3	2.65 E+7	1.28 E+6	3.68 E+7
4	250	7.00 E+2	2.11 E+7	2.50 E+5	4.43 E+7
5	250	2.83 E+2	1.83 E+7	4.67 E+5	4.92 E+7
6	250	2.83 E+2	2.61 E+7	2.67 E+5	4.24 E+7
7	500	1.67 E+2	2.51 E+7	5.5 E+5	4.04 E+7
8	500	1.33 E+2	2.94 E+7	5.17 E+5	5.79 E+7
9	500	6.67 E+1	2.65 E+7	5.50 E+5	5.40 E+7

Table 10: Viable and total cell mean counts for control planktonic experiments.

Exp.	Conc. ($\mu\text{g ml}^{-1}$)	Control	
		Viable Cells (cfu ml^{-1})	Total Cells (cells ml^{-1})
10	0	5.57 E+7	3.78 E+7
11	0	5.33 E+7	2.74 E+7
12	0	1.38 E+8	4.20 E+7

Table 11: Viable and total cell mean counts for biofilms exposed to levofloxacin and ciprofloxacin.

Exp.	Conc. ($\mu\text{g ml}^{-1}$)	Levofloxacin		Ciprofloxacin	
		Viable Cells (cfu m^{-2})	Total Cells (cells m^{-2})	Viable Cells (cfu m^{-2})	Total Cells (cells m^{-2})
15	500	7.75 E+6	1.72 E+8	1.63 E+6	8.17 E+7
16	100	2.06 E+7	4.36 E+8	2.62 E+7	4.82 E+8
17	250	1.63 E+8	7.47 E+8	2.09 E+7	5.02 E+8
18	500	6.13 E+6	1.91 E+8	9.12 E+6	3.34 E+8
19	100	3.36 E+7	1.45 E+8	1.21 E+7	1.25 E+8
20	250	3.07 E+6	1.03 E+8	2.09 E+6	1.36 E+8

Table 12: Viable and total cell mean counts for control biofilm experiments.

Exp.	Conc. ($\mu\text{g ml}^{-1}$)	Control	
		Viable Cells (cfu m^{-2})	Total Cells (cells m^{-2})
21a	0	3.16 E+8	2.73 E+8
21b	0	2.96 E+8	3.05 E+8

Table 13: Total cell mean counts for germanium coupons used in biofilm experiments for cryoembedding and examination by confocal microscopy.

Exp.	Total Cells for Coupon #1 (cells m^{-2})	Total Cells for Coupon #2 (cells m^{-2})
15	5.55 E+7	3.12 E+8
17	1.51 E+8	2.31 E+8
19	1.36 E+8	1.17 E+8
20	8.82 E+7	9.07 E+7
21	9.88 E+7	1.02 E+8

APPENDIX C

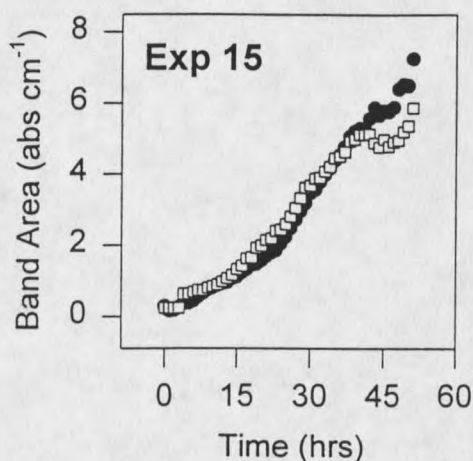
IRE Lateral Colonization Data

Figure 54: Lateral colonization of the IRE surface in sample chamber #1 (●) and sample chamber #2 (□) monitored by the Amide II band (1580-1500 cm^{-1}) area for experiment #15.

Table 14: Experiment #15 lateral colonization data for sample chambers #1 (S #1) and #2 (S #2).

Time (hrs)	Band Area (abs cm^{-1})		Time --cont.	Band Area --cont.		Time --cont.	Band Area --cont.	
	S #1	S #2		S #1	S #2		S #1	S #2
0	0.283	0.243	6	0.450	0.690	12	0.936	0.976
1	0.160	0.243	7	0.565	0.743	13	0.959	1.067
2	0.177	0.243	8	0.653	0.736	14	1.064	1.160
3	0.269	0.243	9	0.751	0.805	15	1.117	1.319
4	0.372	0.634	10	0.804	0.846	16	1.234	1.424
5	0.378	0.644	11	0.878	0.890	17	1.327	1.623

Table 14: Continued.

Time (hrs)	Band Area (abs cm ⁻¹)		Time --cont.	Band Area --cont.		Time --cont.	Band Area --cont.	
18	1.437	1.655	30	3.463	3.677	42	5.572	5.127
19	1.482	1.924	31	3.560	3.873	43	5.873	4.885
20	1.575	1.986	32	3.792	3.902	44	5.674	4.759
21	1.692	2.143	33	3.984	4.077	45	5.778	4.980
22	1.781	2.187	34	4.160	4.207	46	5.770	4.778
23	1.861	2.394	35	4.386	4.448	47	5.884	4.899
24	2.039	2.438	36	4.539	4.494	48	6.398	4.955
25	2.227	2.584	37	4.768	4.625	49	6.523	5.195
26	2.469	2.800	38	5.065	4.927	50	6.495	5.363
27	2.713	3.041	39	5.204	4.984	51	7.248	5.867
28	2.957	3.317	40	5.289	5.113			
29	3.181	3.617	41	5.283	5.114			

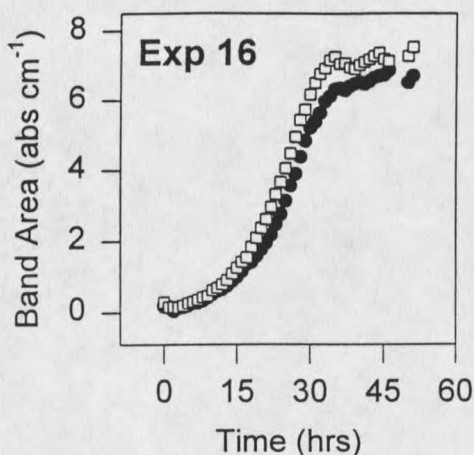


Figure 55: Lateral colonization of the IRE surface in sample chamber #1 (●) and sample chamber #2 (□) monitored by the Amide II band (1580-1500 cm⁻¹) area for experiment #16.

Table 15: Experiment #16 lateral colonization data for sample chambers #1 (S #1) and #2 (S #2).

Time (hrs)	Band Area (abs cm ⁻¹)		Time --cont.	Band Area --cont.		Time --cont.	Band Area --cont.	
	S #1	S #2		S #1	S #2		S #1	S #2
0	0.173	0.283	18	1.411	1.839	36	6.350	7.053
1	0.157	0.154	19	1.591	2.097	37	6.284	7.073
2	0.045	0.157	20	1.771	2.368	38	6.410	6.948
3	0.140	0.142	21	1.950	2.617	39	6.485	6.906
4	0.168	0.195	22	2.177	2.966	40	6.524	7.024
5	0.210	0.263	23	2.469	3.325	41	6.479	7.118
6	0.269	0.305	24	2.762	3.674	42	6.557	7.190
7	0.290	0.359	25	3.130	4.059	43	6.658	7.285
8	0.335	0.427	26	3.559	4.486	44	6.707	7.377
9	0.422	0.469	27	3.896	4.950	45	6.738	7.198
10	0.516	0.621	28	4.380	5.443	46	6.869	7.140
11	0.601	0.690	29	4.841	5.737	47	--	--
12	0.651	0.787	30	5.210	6.167	48	--	--
13	0.744	0.910	31	5.383	6.506	49	--	--
14	0.877	1.088	32	5.631	6.765	50	6.513	7.276
15	0.995	1.252	33	5.969	6.926	51	6.702	7.538
16	1.160	1.417	34	6.122	7.159			
17	1.346	1.534	35	6.317	7.263			

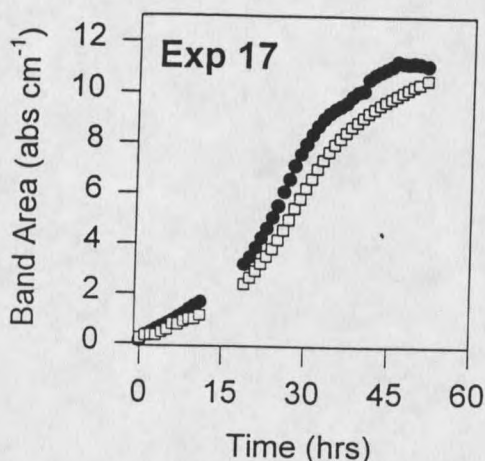


Figure 56: Lateral colonization of the IRE surface in sample chamber #1 (●) and sample chamber #2 (□) monitored by the Amide II band (1580-1500 cm⁻¹) area for experiment #17. Gaps in data were due to a power failure.

Table 16: Experiment #17 lateral colonization data for sample chambers #1 (S #1) and #2 (S #2). ---- indicates time when no data was obtained.

Time (hrs)	Band Area (abs cm ⁻¹)		Time --cont.	Band Area --cont.		Time --cont.	Band Area --cont.	
	S #1	S #2		S #1	S #2		S #1	S #2
0	0.134	0.228	18	--	--	36	9.482	8.190
1	0.354	0.328	19	3.163	2.375	37	9.619	8.427
2	0.467	0.352	20	3.452	2.629	38	9.857	8.654
3	0.593	0.336	21	3.823	2.894	39	10.037	8.844
4	0.702	0.460	22	4.211	3.187	40	10.105	9.032
5	0.810	0.566	23	4.594	3.478	41	10.563	9.208
6	0.929	0.787	24	5.023	3.784	42	10.727	9.370
7	1.080	0.779	25	5.489	4.137	43	10.847	9.527
8	1.203	0.900	26	6.040	4.530	44	10.958	9.662
9	1.372	0.978	27	6.548	4.943	45	11.075	9.784
10	1.527	1.008	28	7.096	5.399	46	11.267	9.914
11	1.652	1.132	29	7.550	5.781	47	11.197	10.031
12	--	--	30	7.956	6.192	48	11.168	10.151
13	--	--	31	8.345	6.573	49	11.214	10.280
14	--	--	32	8.677	6.971	50	11.174	10.378
15	--	--	33	8.962	7.322	51	11.101	10.538
16	--	--	34	9.182	7.655			
17	--	--	35	9.349	7.938			

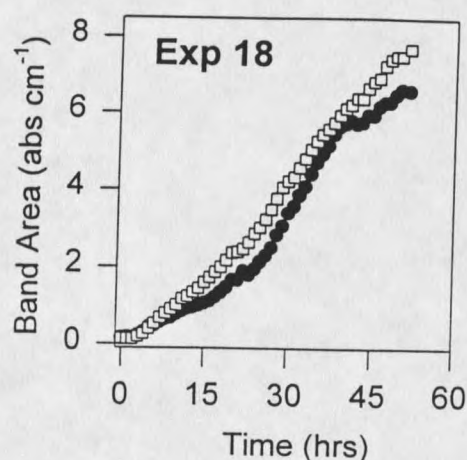


Figure 57: Lateral colonization of the IRE surface in sample chamber #1 (●) and sample chamber #2 (□) monitored by the Amide II band (1580-1500 cm^{-1}) area for experiment #18.

Table 17: Experiment #18 lateral colonization data for sample chambers #1 (S #1) and #2 (S #2).

Time (hrs)	Band Area (abs cm^{-1})		Time --cont.	Band Area --cont.		Time --cont.	Band Area --cont.	
	S #1	S #2		S #1	S #2		S #1	S #2
0	0.162	0.146	18	1.358	2.007	36	4.953	5.458
1	0.178	0.139	19	1.490	2.178	37	5.159	5.599
2	0.153	0.136	20	1.674	2.368	38	5.467	5.762
3	0.239	0.199	21	1.679	2.399	39	5.674	5.961
4	0.320	0.270	22	1.896	2.475	40	5.746	6.138
5	0.456	0.403	23	1.804	2.639	41	5.819	6.235
6	0.489	0.540	24	1.953	2.863	42	5.741	6.409
7	0.577	0.695	25	2.144	3.025	43	5.773	6.405
8	0.658	0.808	26	2.308	3.231	44	5.972	6.679
9	0.687	0.912	27	2.509	3.492	45	5.958	6.853
10	0.792	1.020	28	2.834	3.777	46	6.192	6.942
11	0.861	1.181	29	3.068	4.070	47	6.294	7.267
12	0.927	1.244	30	3.401	4.234	48	6.322	7.433
13	0.963	1.354	31	3.584	4.339	49	6.508	7.520
14	1.006	1.452	32	3.873	4.578	50	6.639	7.521
15	1.072	1.603	33	4.095	4.816	51	6.608	7.717
16	1.128	1.733	34	4.427	5.066			
17	1.235	1.872	35	4.718	5.276			

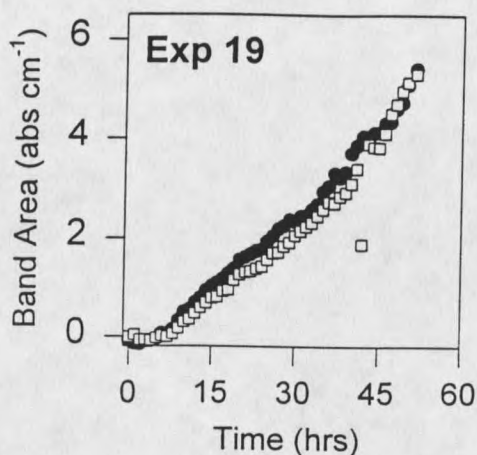


Figure 58: Lateral colonization of the IRE surface in sample chamber #1 (●) and sample chamber #2 (□) monitored by the Amide II band (1580-1500 cm^{-1}) area for experiment #19.

Table 18: Experiment #19 lateral colonization data for sample chambers #1 (S #1) and #2 (S #2).

Time (hrs)	Band Area (abs cm^{-1})		Time --cont.	Band Area --cont.		Time --cont.	Band Area --cont.	
	S #1	S #2		S #1	S #2		S #1	S #2
0	-0.082	-0.007	18	1.288	0.952	36	3.053	2.730
1	-0.145	0.037	19	1.384	1.152	37	3.302	2.712
2	-0.159	-0.064	20	1.571	1.269	38	3.217	2.843
3	-0.094	-0.061	21	1.622	1.319	39	3.330	2.924
4	-0.088	-0.077	22	1.681	1.341	40	3.705	3.103
5	-0.049	-0.030	23	1.746	1.390	41	3.871	3.402
6	0.061	0.017	24	1.782	1.438	42	4.053	1.890
7	0.019	-0.010	25	1.938	1.571	43	3.952	3.896
8	0.155	0.056	26	2.048	1.703	44	4.124	3.835
9	0.326	0.164	27	2.193	1.723	45	4.134	3.821
10	0.508	0.297	28	2.252	1.844	46	4.321	4.109
11	0.556	0.351	29	2.375	1.920	47	4.349	4.509
12	0.694	0.468	30	2.337	2.055	48	4.601	4.702
13	0.792	0.582	31	2.442	2.148	49	4.732	4.969
14	0.938	0.683	32	2.455	2.235	50	5.098	5.143
15	1.036	0.785	33	2.578	2.318	51	5.410	5.307
16	1.104	0.808	34	2.685	2.448			
17	1.182	0.922	35	2.934	2.586			

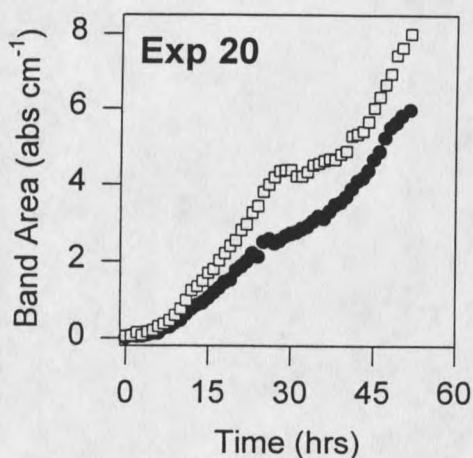


Figure 59: Lateral colonization of the IRE surface in sample chamber #1 (●) and sample chamber #2 (□) monitored by the Amide II band (1580-1500 cm^{-1}) area for experiment #20.

Table 19: Experiment #20 lateral colonization data for sample chambers #1 (S #1) and #2 (S #2).

Time (hrs)	Band Area (abs cm^{-1})		Time --cont.	Band Area --cont.		Time --cont.	Band Area --cont.	
	S #1	S #2		S #1	S #2		S #1	S #2
0	0.013	0.054	18	1.460	2.202	36	3.112	4.616
1	0.063	0.065	19	1.516	2.397	37	3.296	4.670
2	0.055	0.148	20	1.761	2.548	38	3.463	4.646
3	0.058	0.083	21	1.864	2.810	39	3.526	4.791
4	0.088	0.166	22	1.992	2.959	40	3.677	4.868
5	0.131	0.211	23	2.204	3.266	41	3.880	5.274
6	0.138	0.310	24	2.115	3.433	42	4.076	5.319
7	0.261	0.378	25	2.497	3.800	43	4.161	5.402
8	0.294	0.466	26	2.571	3.994	44	4.358	5.652
9	0.402	0.601	27	2.467	4.186	45	4.651	6.013
10	0.480	0.760	28	2.540	4.344	46	4.866	6.316
11	0.630	0.987	29	2.646	4.370	47	5.225	6.649
12	0.767	1.245	30	2.718	4.377	48	5.493	6.937
13	0.866	1.348	31	2.749	4.214	49	5.626	7.426
14	0.928	1.488	32	2.842	4.206	50	5.810	7.639
15	1.044	1.696	33	2.897	4.321	51	5.964	8.006
16	1.182	1.797	34	3.032	4.498			
17	1.308	2.026	35	3.157	4.545			

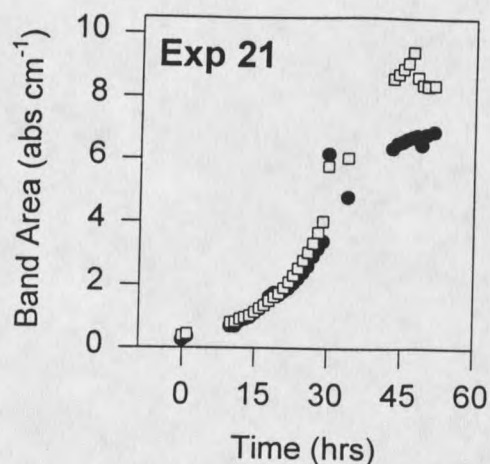


Figure 60: Lateral colonization of the IRE surface in sample chamber #1 (●) and sample chamber #2 (□) monitored by the Amide II band (1580-1500 cm^{-1}) area for experiment #21. Gaps in data were due to a power failure.

Table 20: Experiment #21 lateral colonization data for sample chambers #1 (S #1) and #2 (S #2). ---- indicates time when no data was obtained.

Time (hrs)	Band Area (abs cm^{-1})		Time --cont.	Band Area --cont.		Time --cont.	Band Area --cont.	
	S #1	S #2		S #1	S #2		S #1	S #2
0	0.262	0.385	18	1.634	1.500	36	--	--
1	0.361	0.439	19	1.764	1.632	37	--	--
2	--	--	20	1.686	1.746	38	--	--
3	--	--	21	1.822	1.918	39	--	--
4	--	--	22	1.912	2.110	40	--	--
5	--	--	23	2.066	2.318	41	--	--
6	--	--	24	2.225	2.543	42	--	--
7	--	--	25	2.411	2.797	43	6.348	8.585
8	--	--	26	2.611	3.052	44	6.506	8.726
9	--	--	27	2.925	3.346	45	6.568	8.901
10	0.699	0.813	28	3.164	3.683	46	6.656	9.091
11	0.697	0.837	29	3.396	4.034	47	6.716	9.449
12	0.859	0.918	30	6.176	5.778	48	6.778	8.642
13	0.918	0.975	31	--	--	49	6.489	8.403
14	0.974	1.032	32	--	--	50	6.813	8.361
15	1.114	1.152	33	--	--	51	6.903	8.390
16	1.235	1.270	34	4.797	6.052			
17	1.368	1.366	35	--	--			

APPENDIX D

FTIR Delivery Data—Uncolonized Experiments

Table 21: FTIR delivery data for uncolonized IRE experiment #22, levofloxacin 100 $\mu\text{g ml}^{-1}$, using band 995-965 cm^{-1} .

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	805.2	0.019606	1573.8	0.019050
36.6	0.010435	841.8	0.013242	1610.4	0.017103
73.2	0.014220	878.4	0.019867	1647.0	0.021629
109.8	0.014920	915.0	0.021444	1683.6	0.021798
146.4	0.012644	951.6	0.017929	1720.2	0.019648
183.0	0.020137	988.2	0.020205	1756.3	0.016116
219.6	0.018595	1024.8	0.019893	1793.4	0.010098
256.2	0.016049	1061.4	0.017575	1830.0	0.008008
292.4	0.015754	1098.0	0.020575	1866.6	0.010326
329.4	0.017659	1134.6	0.018165	1903.2	0.011177
366.0	0.014346	1171.2	0.020162	1939.8	0.008884
402.6	0.015375	1207.8	0.013815	1976.4	0.006752
439.2	0.021039	1244.4	0.021562	2013.0	0.004619
475.8	0.013428	1281.0	0.018906	2049.6	0.002807
512.4	0.013925	1317.6	0.013512	2086.2	-0.001804
585.6	0.021646	1354.2	0.016942	2122.8	0.005125
622.2	0.016470	1390.8	0.015838	2159.4	0.010123
658.8	0.016041	1427.4	0.019404	2196.0	0.004257
695.4	0.018291	1464.0	0.020508	2232.6	0.006474
732.0	0.013883	1500.6	0.022211	2269.2	0.003228
768.6	0.013133	1537.2	0.013664	2305.8	0.003625

Experiment #22--continued

Uncolonized IRE--Levofloxacin 100 $\mu\text{g ml}^{-1}$

Table 21: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
2342.4	0.004923	3294.0	0.002200	4245.6	0.006819
2379.0	0.007578	3330.6	0.005454	4282.2	-0.000464
2415.6	-0.000025	3367.2	0.002377	4318.8	0.000034
2452.2	0.000329	3403.8	0.003026	4355.4	0.003110
2488.8	0.006187	3440.4	0.006389	4392.0	0.001003
2525.4	0.007974	3477.0	0.001028	4428.6	-0.000767
2562.0	0.003388	3513.6	0.007089	4465.2	0.004366
2598.6	0.007544	3550.2	0.001795	4501.8	0.000455
2635.2	0.008572	3586.8	0.006937	4538.4	0.003532
2671.8	0.005251	3623.4	0.001644	4575.0	0.005403
2708.4	0.006120	3660.0	0.004636	4611.6	-0.000480
2745.0	0.005504	3696.6	0.003751	4648.2	-0.002394
2781.6	0.007401	3733.2	0.001736	4684.8	0.005293
2818.2	0.004754	3769.8	0.000818	4721.4	0.002588
2854.8	0.004206	3806.4	0.004771	4758.0	0.001146
2891.4	0.001829	3843.0	0.011666	4794.6	0.000346
2928.0	0.006372	3879.6	0.006111	4831.2	0.002571
2964.6	0.007224	3916.2	0.004299	4867.8	-0.000371
3001.2	0.001930	3952.8	0.003270	4904.4	-0.000169
3037.8	0.005057	3989.4	0.003667	4941.0	0.003186
3074.4	0.007316	4026.0	0.001037	4977.6	-0.003018
3111.0	0.008699	4062.6	0.002815	5014.2	0.000346
3147.6	0.010267	4099.2	0.005816	5050.8	0.001821
3184.2	0.007645	4135.8	0.006634	5087.4	0.004375
3220.8	0.002967	4172.4	0.007637	5124.0	-0.000110
3257.4	0.005900	4209.0	0.001475	5160.6	-0.000868

Experiment #22

Uncolonized IRE--Ciprofloxacin 100 $\mu\text{g ml}^{-1}$ **Table 22: FTIR delivery data for uncolonized IRE experiment #22, ciprofloxacin 100 $\mu\text{g ml}^{-1}$, using band 1350-1325 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.010417	2305.8	-0.000165
36.6	0.005350	1207.8	0.006130	2342.4	0.001418
73.2	0.012395	1244.4	0.011087	2379.0	0.002703
109.8	0.015878	1281.0	0.011772	2415.6	0.003246
146.4	0.016106	1317.6	0.007911	2452.2	-0.000071
183.0	0.014767	1354.2	0.009377	2488.8	0.000323
219.6	0.015452	1390.8	0.007281	2525.4	0.000607
256.2	0.019006	1427.4	0.010401	2562.0	0.002971
292.4	0.012300	1464.0	0.008597	2598.6	-0.000158
329.4	0.013080	1500.6	0.007762	2635.2	0.001245
366.0	0.011615	1537.2	0.006666	2671.8	0.002624
402.6	0.011765	1573.8	0.007115	2708.4	0.001844
439.2	0.013916	1610.4	0.010590	2745.0	0.001868
475.8	0.009944	1647.0	0.007399	2781.6	0.001734
512.4	0.014980	1683.6	0.009014	2818.2	0.001994
585.6	0.014018	1720.2	0.008849	2854.8	-0.000221
622.2	0.009487	1756.3	0.005114	2891.4	0.003270
658.8	0.011765	1793.4	0.000142	2928.0	0.002175
695.4	0.011118	1830.0	-0.004294	2964.6	0.004941
732.0	0.011111	1866.6	-0.003065	3001.2	0.001474
768.6	0.009267	1903.2	-0.002277	3037.8	0.002293
805.2	0.007045	1939.8	-0.001852	3074.4	0.001056
841.8	0.011260	1976.4	0.002214	3111.0	-0.000378
878.4	0.010110	2013.0	-0.001269	3147.6	0.003207
915.0	0.008825	2049.6	0.004736	3184.2	-0.000583
951.6	0.010189	2086.2	0.004783	3220.8	0.002608
988.2	0.008857	2122.8	0.000331	3257.4	0.001986
1024.8	0.009708	2159.4	0.003885	3294.0	0.002206
1061.4	0.011016	2196.0	0.002979	3330.6	0.002789
1098.0	0.010433	2232.6	0.001332	3367.2	-0.000173
1134.6	0.009125	2269.2	0.002301	3403.8	0.001568

Experiment #22--continued

Uncolonized IRE--Ciprofloxacin 100 $\mu\text{g ml}^{-1}$

Table 22: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.004499	4062.6	0.002435	4684.8	0.001505
3477.0	0.002080	4099.2	-0.000725	4721.4	0.000630
3513.6	0.003412	4135.8	0.001568	4758.0	-0.000646
3550.2	0.003412	4172.4	0.002671	4794.6	0.004436
3586.8	0.002301	4209.0	0.003049	4831.2	0.005169
3623.4	0.001206	4245.6	0.001986	4867.8	-0.000528
3660.0	0.003097	4282.2	0.001623	4904.4	0.000213
3696.6	0.004090	4318.8	0.001001	4941.0	0.001686
3733.2	0.001371	4355.4	0.000764	4977.6	0.001206
3769.8	0.003987	4392.0	0.001253	5014.2	0.001954
3806.4	0.001040	4428.6	-0.001198	5050.8	0.001954
3843.0	-0.000292	4465.2	0.003711	5087.4	0.005272
3879.6	0.000410	4501.8	0.002931	5124.0	0.003814
3916.2	0.002065	4538.4	0.003380	5160.6	0.003814
3952.8	0.001812	4575.0	-0.000110		
3989.4	0.001812	4611.6	0.004224		
4026.0	0.001631	4648.2	0.001072		

Experiment #23

Uncolonized IRE--Levofloxacin 250 $\mu\text{g ml}^{-1}$ **Table 23: FTIR delivery data for uncolonized IRE experiment #23, levofloxacin 250 $\mu\text{g ml}^{-1}$, using band 995-965 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.027698	2305.8	0.008264
36.6	0.005739	1207.8	0.032199	2342.4	0.008679
73.2	0.023404	1244.4	0.026278	2379.0	0.003820
109.8	0.027166	1281.0	0.039059	2415.6	0.002957
146.4	0.032075	1317.6	0.027714	2452.2	0.005158
183.0	0.026701	1354.2	0.028703	2488.8	-0.000116
219.6	0.027415	1390.8	0.032374	2525.4	0.005697
256.2	0.029816	1427.4	0.027615	2562.0	0.005108
292.4	0.025954	1464.0	0.030978	2598.6	0.004427
329.4	0.027922	1500.6	0.029218	2635.2	0.002998
366.0	0.028055	1537.2	0.027474	2671.8	0.005672
402.6	0.033420	1573.8	0.031452	2708.4	-0.000066
439.2	0.024783	1610.4	0.027050	2745.0	0.000174
475.8	0.033927	1647.0	0.031186	2781.6	0.004310
512.4	0.035754	1683.6	0.028595	2818.2	0.001570
585.6	0.022034	1720.2	0.033893	2854.8	0.001038
622.2	0.030156	1756.3	0.032058	2891.4	0.001927
658.8	0.027316	1793.4	0.011013	2928.0	0.002865
695.4	0.034425	1830.0	0.007400	2964.6	0.001296
732.0	0.029342	1866.6	0.006669	3001.2	0.002899
768.6	0.030505	1903.2	0.008505	3037.8	0.003563
805.2	0.030115	1939.8	0.005265	3074.4	0.005058
841.8	0.026286	1976.4	0.001918	3111.0	0.004817
878.4	0.032507	2013.0	0.003654	3147.6	-0.004269
915.0	0.031003	2049.6	0.003023	3184.2	0.002732
951.6	0.033694	2086.2	0.001694	3220.8	0.003106
988.2	0.032988	2122.8	0.004842	3257.4	0.004468
1024.8	0.030405	2159.4	0.007109	3294.0	0.006951
1061.4	0.028703	2196.0	0.008729	3330.6	0.006993
1098.0	0.031825	2232.6	0.005830	3367.2	0.003571
1134.6	0.026469	2269.2	0.006262	3403.8	0.001827

Experiment #23--continued

Uncolonized IRE--Levofloxacin 250 $\mu\text{g ml}^{-1}$

Table 23: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.004809	4062.6	0.005332	4684.8	0.004344
3477.0	0.004261	4099.2	0.003339	4721.4	0.004369
3513.6	0.005432	4135.8	0.009127	4758.0	-0.001221
3550.2	0.002126	4172.4	0.000324	4794.6	0.002899
3586.8	0.003937	4209.0	0.002600	4831.2	0.004510
3623.4	0.004144	4245.6	0.000963	4867.8	0.009360
3660.0	0.009269	4282.2	-0.000191	4904.4	0.002691
3696.6	0.001744	4318.8	0.002666	4941.0	0.009219
3733.2	0.003937	4355.4	0.004867	4977.6	0.003048
3769.8	0.005224	4392.0	0.006121	5014.2	0.004011
3806.4	0.004003	4428.6	0.002475	5050.8	0.001528
3843.0	0.002550	4465.2	0.002857	5087.4	0.005299
3879.6	-0.001653	4501.8	0.006511	5124.0	0.001030
3916.2	-0.001528	4538.4	0.002267	5160.6	0.001445
3952.8	0.002093	4575.0	0.003305		
3989.4	-0.000241	4611.6	-0.000963		
4026.0	0.001428	4648.2	0.001171		

Experiment #23

Uncolonized IRE--Ciprofloxacin 250 $\mu\text{g ml}^{-1}$ **Table 24: FTIR delivery data for uncolonized IRE experiment #23, ciprofloxacin 250 $\mu\text{g ml}^{-1}$, using band 1350-1325 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.031421	2305.8	0.002640
36.6	0.004039	1207.8	0.028255	2342.4	0.001658
73.2	0.029787	1244.4	0.029685	2379.0	0.001768
109.8	0.032089	1281.0	0.034219	2415.6	0.000212
146.4	0.027438	1317.6	0.029614	2452.2	0.003276
183.0	0.029473	1354.2	0.026676	2488.8	0.000676
219.6	0.033873	1390.8	0.032286	2525.4	0.004235
256.2	0.033810	1427.4	0.029206	2562.0	0.004306
292.4	0.031162	1464.0	0.031751	2598.6	0.001399
329.4	0.033315	1500.6	0.033629	2635.2	0.002224
366.0	0.031437	1537.2	0.031594	2671.8	0.001564
402.6	0.027721	1573.8	0.033676	2708.4	0.002231
439.2	0.031547	1610.4	0.032482	2745.0	0.003465
475.8	0.033158	1647.0	0.034171	2781.6	0.001029
512.4	0.030864	1683.6	0.028805	2818.2	0.003709
585.6	0.028868	1720.2	0.030510	2854.8	0.004062
622.2	0.030777	1756.3	0.030722	2891.4	-0.000189
658.8	0.033873	1793.4	0.017168	2928.0	0.001894
695.4	0.029072	1830.0	-0.000008	2964.6	0.000660
732.0	0.032380	1866.6	-0.000519	3001.2	-0.002569
768.6	0.030314	1903.2	-0.002098	3037.8	0.003245
805.2	0.031154	1939.8	0.002247	3074.4	0.002066
841.8	0.030117	1976.4	0.002585	3111.0	-0.000102
878.4	0.032325	2013.0	0.002601	3147.6	-0.000542
915.0	0.032058	2049.6	0.001147	3184.2	0.001147
951.6	0.030691	2086.2	-0.000809	3220.8	0.004518
988.2	0.029520	2122.8	0.001477	3257.4	-0.001807
1024.8	0.030384	2159.4	-0.000966	3294.0	0.002294
1061.4	0.032380	2196.0	-0.000385	3330.6	-0.000259
1098.0	0.032105	2232.6	0.003457	3367.2	-0.000809
1134.6	0.029221	2269.2	0.002263	3403.8	-0.002098

Experiment #23--continued

Uncolonized IRE--Ciprofloxacin 250 $\mu\text{g ml}^{-1}$

Table 24: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	-0.000173	4062.6	0.003041	4684.8	0.001729
3477.0	0.001571	4099.2	0.001556	4721.4	0.004157
3513.6	0.003379	4135.8	0.001540	4758.0	0.000762
3550.2	0.002247	4172.4	0.002585	4794.6	0.005508
3586.8	0.002656	4209.0	-0.000181	4831.2	-0.000031
3623.4	0.002656	4245.6	-0.001430	4867.8	0.001611
3660.0	-0.000283	4282.2	0.001988	4904.4	0.001124
3696.6	0.005422	4318.8	0.002671	4941.0	0.002719
3733.2	0.002365	4355.4	-0.000086	4977.6	0.001972
3769.8	0.002459	4392.0	0.004070	5014.2	0.002176
3806.4	-0.000778	4428.6	0.005359	5050.8	0.001674
3843.0	0.002271	4465.2	0.000361	5087.4	0.002530
3879.6	0.003127	4501.8	0.003261	5124.0	0.001516
3916.2	0.000299	4538.4	0.002946	5160.6	0.002915
3952.8	0.002239	4575.0	0.003614		
3989.4	0.000369	4611.6	0.005539		
4026.0	0.001831	4648.2	0.001721		

Experiment #24

Uncolonized IRE--Levofloxacin 500 $\mu\text{g ml}^{-1}$ **Table 25: FTIR delivery data for uncolonized IRE experiment #24, levofloxacin 500 $\mu\text{g ml}^{-1}$, using band 995-965 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.059919	2305.8	0.000372
36.6	0.009624	1207.8	0.056507	2342.4	0.000958
73.2	0.048477	1244.4	0.055565	2379.0	0.002883
109.8	0.049906	1281.0	0.058523	2415.6	0.000826
146.4	0.046701	1317.6	0.062199	2452.2	-0.000958
183.0	0.050369	1354.2	0.055169	2488.8	0.002660
219.6	0.053830	1390.8	0.064108	2525.4	0.003057
256.2	0.054557	1427.4	0.058903	2562.0	0.005552
292.4	0.058630	1464.0	0.059878	2598.6	0.003139
329.4	0.050675	1500.6	0.058672	2635.2	0.002652
366.0	0.055541	1537.2	0.058886	2671.8	0.000727
402.6	0.050138	1573.8	0.060836	2708.4	0.004172
439.2	0.058102	1610.4	0.065752	2745.0	-0.002536
475.8	0.047420	1647.0	0.062009	2781.6	-0.001884
512.4	0.058391	1683.6	0.057639	2818.2	0.007427
585.6	0.052922	1720.2	0.057333	2854.8	0.003371
622.2	0.054161	1756.3	0.060943	2891.4	0.002454
658.8	0.052079	1793.4	0.027304	2928.0	0.001214
695.4	0.057482	1830.0	0.012698	2964.6	0.000578
732.0	0.055574	1866.6	0.006774	3001.2	-0.001148
768.6	0.057152	1903.2	0.001809	3037.8	0.002916
805.2	0.055598	1939.8	0.000884	3074.4	0.000768
841.8	0.055598	1976.4	0.005907	3111.0	0.001198
878.4	0.057755	2013.0	0.004692	3147.6	0.003437
915.0	0.060919	2049.6	0.004651	3184.2	0.000900
951.6	0.061513	2086.2	-0.001958	3220.8	0.004263
988.2	0.057251	2122.8	0.002536	3257.4	0.000587
1024.8	0.056962	2159.4	0.002586	3294.0	-0.001586
1061.4	0.055524	2196.0	-0.003073	3330.6	-0.000512
1098.0	0.056548	2232.6	0.005733	3367.2	0.005048
1134.6	0.054805	2269.2	0.001661	3403.8	-0.001090

Experiment #24--continued

Uncolonized IRE--Levofloxacin 500 $\mu\text{g ml}^{-1}$

Table 25: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.000264	4062.6	-0.002858	4684.8	-0.002049
3477.0	-0.001917	4099.2	-0.001454	4721.4	-0.001148
3513.6	0.001347	4135.8	-0.000083	4758.0	0.000826
3550.2	0.002974	4172.4	-0.000132	4794.6	-0.003404
3586.8	0.000950	4209.0	-0.001801	4831.2	0.000297
3623.4	0.003635	4245.6	-0.001603	4867.8	-0.000570
3660.0	-0.000149	4282.2	0.000198	4904.4	-0.003561
3696.6	0.001429	4318.8	-0.001388	4941.0	-0.004098
3733.2	-0.004370	4355.4	0.004420	4977.6	-0.003668
3769.8	-0.001892	4392.0	-0.000520	5014.2	-0.002520
3806.4	0.002404	4428.6	0.002074	5050.8	0.003271
3843.0	0.001000	4465.2	-0.004568	5087.4	-0.002503
3879.6	0.000719	4501.8	0.001330	5124.0	0.001553
3916.2	0.005221	4538.4	0.000223	5160.6	0.001429
3952.8	-0.000289	4575.0	-0.003833	5197.2	0.001355
3989.4	-0.000339	4611.6	-0.002148		
4026.0	0.002024	4648.2	-0.001537		

Experiment #24

Uncolonized IRE--Ciprofloxacin 500 $\mu\text{g ml}^{-1}$ **Table 26: FTIR delivery data for uncolonized IRE experiment #24, ciprofloxacin 500 $\mu\text{g ml}^{-1}$, using band 1350-1325 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.050479	2305.8	0.005075
36.6	0.020027	1207.8	0.046759	2342.4	0.002796
73.2	0.046054	1244.4	0.048873	2379.0	0.007848
109.8	0.047659	1281.0	0.048443	2415.6	0.005835
146.4	0.050644	1317.6	0.046116	2452.2	0.003454
183.0	0.047503	1354.2	0.052594	2488.8	0.003399
219.6	0.050730	1390.8	0.047761	2525.4	0.000298
256.2	0.048051	1427.4	0.047996	2562.0	0.006775
292.4	0.044996	1464.0	0.047918	2598.6	0.007331
329.4	0.046015	1500.6	0.049876	2635.2	0.003752
366.0	0.046195	1537.2	0.047597	2671.8	0.004801
402.6	0.048591	1573.8	0.048028	2708.4	0.002232
439.2	0.047910	1610.4	0.047471	2745.0	0.002334
475.8	0.047056	1647.0	0.049328	2781.6	0.005279
512.4	0.046884	1683.6	0.049328	2818.2	0.004088
585.6	0.046782	1720.2	0.051239	2854.8	0.005577
622.2	0.047377	1756.3	0.047824	2891.4	0.004598
658.8	0.048740	1793.4	0.038206	2928.0	0.004284
695.4	0.048216	1830.0	0.005122	2964.6	0.003313
732.0	0.047346	1866.6	0.003814	3001.2	0.003759
768.6	0.048028	1903.2	0.004778	3037.8	0.002671
805.2	0.049852	1939.8	0.001104	3074.4	0.002718
841.8	0.042819	1976.4	0.004276	3111.0	0.002530
878.4	0.050189	2013.0	0.004629	3147.6	0.004699
915.0	0.046007	2049.6	0.004002	3184.2	0.005193
951.6	0.048905	2086.2	0.003368	3220.8	0.005161
988.2	0.052218	2122.8	0.006211	3257.4	0.003587
1024.8	0.048270	2159.4	0.004598	3294.0	0.005984
1061.4	0.047730	2196.0	0.003102	3330.6	0.006321
1098.0	0.046892	2232.6	0.005357	3367.2	0.003799
1134.6	0.050244	2269.2	0.006180	3403.8	0.004739

Experiment #24--continued

Uncolonized IRE--Ciprofloxacin 500 $\mu\text{g ml}^{-1}$

Table 26: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.005185	4062.6	0.004308	4684.8	0.006383
3477.0	0.003250	4099.2	0.003047	4721.4	0.003861
3513.6	0.004629	4135.8	0.005169	4758.0	0.003172
3550.2	0.007801	4172.4	0.005686	4794.6	0.003877
3586.8	0.005553	4209.0	0.003673	4831.2	0.003070
3623.4	0.005553	4245.6	0.005044	4867.8	0.004237
3660.0	0.004229	4282.2	0.002420	4904.4	0.005718
3696.6	0.004386	4318.8	0.003869	4941.0	0.001598
3733.2	0.005569	4355.4	0.003015	4977.6	0.004715
3769.8	0.002044	4392.0	0.005874	5014.2	0.002491
3806.4	0.003877	4428.6	0.003752	5050.8	0.001433
3843.0	0.003337	4465.2	0.004065	5087.4	0.003963
3879.6	0.006375	4501.8	0.005404	5124.0	0.002780
3916.2	0.004049	4538.4	0.005569	5160.6	0.002389
3952.8	0.006462	4575.0	0.002498	5197.2	0.006015
3989.4	0.002961	4611.6	0.004284		
4026.0	0.005224	4648.2	0.001637		

Experiment #25

Uncolonized IRE--Levofloxacin 100 $\mu\text{g ml}^{-1}$ **Table 27: FTIR delivery data for uncolonized IRE experiment #25, levofloxacin 100 $\mu\text{g ml}^{-1}$, using band 995-965 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.021153	2305.8	-0.006038
36.6	0.001711	1207.8	0.020016	2342.4	-0.001271
73.2	0.010996	1244.4	0.024068	2379.0	-0.003845
109.8	0.007491	1281.0	0.023927	2415.6	0.002774
146.4	0.011337	1317.6	0.020821	2452.2	0.005548
183.0	0.011478	1354.2	0.019816	2488.8	0.004991
219.6	0.011420	1390.8	0.023653	2525.4	0.003455
256.2	0.012358	1427.4	0.027175	2562.0	-0.004875
292.4	0.015680	1464.0	0.022216	2598.6	-0.000341
329.4	0.012441	1500.6	0.023554	2635.2	0.000357
366.0	0.014999	1537.2	0.023080	2671.8	0.005133
402.6	0.015855	1573.8	0.023371	2708.4	-0.005000
439.2	0.017790	1610.4	0.025090	2745.0	0.001063
475.8	0.020248	1647.0	0.021602	2781.6	-0.000922
512.4	0.018645	1683.6	0.026876	2818.2	-0.004651
585.6	0.013737	1720.2	0.025090	2854.8	-0.003472
622.2	0.023628	1756.3	0.019368	2891.4	0.000324
658.8	0.014750	1793.4	0.015788	2928.0	-0.006686
695.4	0.021643	1830.0	0.008646	2964.6	-0.000490
732.0	0.019991	1866.6	0.007425	3001.2	-0.004900
768.6	0.013280	1903.2	0.004991	3037.8	-0.002782
805.2	0.021502	1939.8	0.002392	3074.4	-0.003613
841.8	0.020813	1976.4	0.003721	3111.0	-0.001977
878.4	0.017441	2013.0	0.001321	3147.6	0.002973
915.0	0.021785	2049.6	0.002442	3184.2	-0.003347
951.6	0.022416	2086.2	0.002442	3220.8	-0.001977
988.2	0.024251	2122.8	0.003505	3257.4	0.000698
1024.8	0.023985	2159.4	-0.000249	3294.0	-0.002940
1061.4	0.023711	2196.0	-0.004011	3330.6	-0.002608
1098.0	0.022034	2232.6	0.001304	3367.2	-0.004418
1134.6	0.024434	2269.2	0.000257	3403.8	-0.003073

Experiment #25--continued

Uncolonized IRE--Levofloxacin 100 $\mu\text{g ml}^{-1}$

Table 27: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	-0.001620	4062.6	0.000324	4684.8	-0.000822
3477.0	-0.002923	4099.2	-0.003638	4721.4	-0.000440
3513.6	-0.003962	4135.8	-0.006827	4758.0	-0.002051
3550.2	-0.004327	4172.4	-0.002492	4794.6	0.000025
3586.8	0.003090	4209.0	-0.005564	4831.2	-0.002483
3623.4	0.001229	4245.6	-0.002791	4867.8	-0.002367
3660.0	0.001229	4282.2	0.005340	4904.4	-0.001636
3696.6	0.000282	4318.8	0.000689	4941.0	-0.010124
3733.2	0.002674	4355.4	-0.003073	4977.6	-0.010888
3769.8	-0.002666	4392.0	0.004335	5014.2	-0.008496
3806.4	-0.001869	4428.6	0.002325	5050.8	-0.003912
3843.0	-0.001553	4465.2	-0.005897	5087.4	-0.007259
3879.6	-0.002450	4501.8	0.004128	5124.0	-0.001420
3916.2	0.001063	4538.4	-0.004518	5160.6	-0.007167
3952.8	-0.003347	4575.0	0.002060	5197.2	-0.001943
3989.4	-0.003413	4611.6	-0.001545		
4026.0	-0.002325	4648.2	-0.006121		

Experiment #25

Uncolonized IRE--Ciprofloxacin 100 $\mu\text{g ml}^{-1}$ **Table 28: FTIR delivery data for uncolonized IRE experiment #25, ciprofloxacin 100 $\mu\text{g ml}^{-1}$, using band 1350-1325 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.021137	2305.8	0.008568
36.6	0.003905	1207.8	0.021239	2342.4	0.004031
73.2	0.034361	1244.4	0.021216	2379.0	0.002024
109.8	0.033618	1281.0	0.018789	2415.6	0.006007
146.4	0.026188	1317.6	0.019651	2452.2	0.001858
183.0	0.023018	1354.2	0.021990	2488.8	0.001494
219.6	0.023097	1390.8	0.021239	2525.4	0.005944
256.2	0.022077	1427.4	0.018615	2562.0	0.002379
292.4	0.020188	1464.0	0.020465	2598.6	0.006371
329.4	0.023777	1500.6	0.020338	2635.2	0.002585
366.0	0.022457	1537.2	0.016789	2671.8	0.002585
402.6	0.020931	1573.8	0.020623	2708.4	0.004814
439.2	0.023160	1610.4	0.020354	2745.0	0.003944
475.8	0.019319	1647.0	0.021271	2781.6	0.006047
512.4	0.023192	1683.6	0.021271	2818.2	0.006039
585.6	0.021066	1720.2	0.019176	2854.8	0.002403
622.2	0.021105	1756.3	0.017003	2891.4	0.000372
658.8	0.022109	1793.4	0.003162	2928.0	0.006498
695.4	0.020157	1830.0	-0.003083	2964.6	0.004387
732.0	0.023666	1866.6	-0.002174	3001.2	0.003960
768.6	0.017216	1903.2	0.000174	3037.8	0.005897
805.2	0.021603	1939.8	-0.002869	3074.4	0.002103
841.8	0.021824	1976.4	0.001945	3111.0	0.006972
878.4	0.021508	2013.0	0.002822	3147.6	0.005573
915.0	0.020077	2049.6	0.002545	3184.2	0.003075
951.6	0.018995	2086.2	0.004932	3220.8	0.002490
988.2	0.018157	2122.8	0.003770	3257.4	0.003573
1024.8	0.019058	2159.4	0.001557	3294.0	0.003407
1061.4	0.017548	2196.0	0.002909	3330.6	0.004996
1098.0	0.024093	2232.6	0.005446	3367.2	0.002435
1134.6	0.019113	2269.2	0.003352	3403.8	0.004640

Experiment #25--continued

Uncolonized IRE--Ciprofloxacin 100 $\mu\text{g ml}^{-1}$

Table 28: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.002561	4062.6	0.003241	4684.8	0.002925
3477.0	0.003684	4099.2	0.003573	4721.4	0.003889
3513.6	0.003778	4135.8	0.003320	4758.0	0.005336
3550.2	0.006023	4172.4	0.004474	4794.6	0.003533
3586.8	0.002846	4209.0	0.003834	4831.2	0.003731
3623.4	0.003510	4245.6	0.002292	4867.8	0.003636
3660.0	-0.000024	4282.2	0.002229	4904.4	0.002790
3696.6	0.004790	4318.8	0.003597	4941.0	0.003810
3733.2	0.003778	4355.4	0.002640	4977.6	0.003280
3769.8	0.002111	4392.0	0.002071	5014.2	0.003699
3806.4	0.004340	4428.6	0.004363	5050.8	0.000174
3843.0	0.006758	4465.2	0.003059	5087.4	0.004506
3879.6	0.005114	4501.8	0.004964	5124.0	0.006007
3916.2	0.006316	4538.4	0.001423	5160.6	0.004450
3952.8	0.005027	4575.0	0.005834	5197.2	0.001873
3989.4	0.001897	4611.6	0.004600		
4026.0	0.000901	4648.2	0.004988		

Experiment #26

Uncolonized IRE--Levofloxacin 250 $\mu\text{g ml}^{-1}$ **Table 29: FTIR delivery data for uncolonized IRE experiment #26, levofloxacin 250 $\mu\text{g ml}^{-1}$, using band 995-965 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.032482	2305.8	-0.005968
36.6	0.018815	1207.8	0.045171	2342.4	0.006970
73.2	0.041562	1244.4	0.057853	2379.0	0.016357
109.8	0.039195	1281.0	0.032043	2415.6	0.014469
146.4	0.032324	1317.6	0.050676	2452.2	-0.003924
183.0	0.030661	1354.2	0.055452	2488.8	0.006068
219.6	0.040453	1390.8	0.049310	2525.4	0.009155
256.2	0.047588	1427.4	0.026671	2562.0	-0.007582
292.4	0.042266	1464.0	0.031728	2598.6	-0.011787
329.4	0.050610	1500.6	0.048168	2635.2	0.003419
366.0	0.047837	1537.2	0.045486	2671.8	0.003816
402.6	0.038425	1573.8	0.044344	2708.4	-0.003824
439.2	0.049277	1610.4	0.053366	2745.0	0.006274
475.8	0.038740	1647.0	0.044253	2781.6	0.007392
512.4	0.036728	1683.6	0.036968	2818.2	0.003054
585.6	0.039038	1720.2	0.036066	2854.8	0.006879
622.2	0.047597	1756.3	0.032382	2891.4	0.006233
658.8	0.034352	1793.4	0.023691	2928.0	0.015471
695.4	0.046098	1830.0	0.015521	2964.6	0.000679
732.0	0.034030	1866.6	0.012996	3001.2	0.005554
768.6	0.042862	1903.2	0.015901	3037.8	-0.002036
805.2	0.040006	1939.8	-0.006076	3074.4	0.000985
841.8	0.036662	1976.4	-0.003054	3111.0	0.004288
878.4	0.049890	2013.0	0.003352	3147.6	0.006341
915.0	0.050072	2049.6	0.010794	3184.2	-0.006738
951.6	0.040172	2086.2	0.013758	3220.8	0.000654
988.2	0.030627	2122.8	-0.006581	3257.4	0.006084
1024.8	0.039195	2159.4	0.007028	3294.0	-0.007913
1061.4	0.039195	2196.0	0.013178	3330.6	0.003352
1098.0	0.042349	2232.6	0.005629	3367.2	-0.006010
1134.6	0.047398	2269.2	0.000877	3403.8	0.000149

Experiment #26--continued

Uncolonized IRE--Levofloxacin 250 $\mu\text{g ml}^{-1}$

Table 29: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.010537	4062.6	0.004271	4684.8	0.000265
3477.0	0.001093	4099.2	-0.003526	4721.4	0.002723
3513.6	0.014850	4135.8	0.011150	4758.0	0.004246
3550.2	0.006581	4172.4	0.015322	4794.6	-0.001324
3586.8	0.002401	4209.0	0.017035	4831.2	-0.001167
3623.4	0.002574	4245.6	-0.011365	4867.8	-0.015272
3660.0	-0.003352	4282.2	-0.007268	4904.4	0.003228
3696.6	-0.010662	4318.8	0.005215	4941.0	0.007756
3733.2	-0.002781	4355.4	-0.003038	4977.6	0.005041
3769.8	0.005422	4392.0	-0.007028	5014.2	0.017027
3806.4	-0.014569	4428.6	0.011622	5050.8	-0.011084
3843.0	0.013840	4465.2	-0.006415	5087.4	0.014097
3879.6	0.007524	4501.8	-0.016986	5124.0	-0.010802
3916.2	0.000430	4538.4	-0.011498	5160.6	0.010595
3952.8	-0.004056	4575.0	-0.012971	5197.2	0.001382
3989.4	-0.011547	4611.6	-0.001250		
4026.0	-0.010273	4648.2	-0.003129		

Experiment #26

Uncolonized IRE--Ciprofloxacin 250 $\mu\text{g ml}^{-1}$ **Table 30: FTIR delivery data for uncolonized IRE experiment #26, ciprofloxacin 250 $\mu\text{g ml}^{-1}$, using band 1350-1325 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.041292	2305.8	0.001380
36.6	0.013052	1207.8	0.038974	2342.4	0.000797
73.2	0.043351	1244.4	0.040094	2379.0	0.003304
109.8	0.041521	1281.0	0.040906	2415.6	0.004897
146.4	0.041537	1317.6	0.039321	2452.2	0.003091
183.0	0.039557	1354.2	0.042680	2488.8	0.007224
219.6	0.045314	1390.8	0.039825	2525.4	0.002098
256.2	0.043043	1427.4	0.040456	2562.0	0.001278
292.4	0.045748	1464.0	0.040551	2598.6	0.003896
329.4	0.038816	1500.6	0.041103	2635.2	0.001727
366.0	0.043595	1537.2	0.040046	2671.8	0.004259
402.6	0.041426	1573.8	0.042925	2708.4	0.003375
439.2	0.042523	1610.4	0.039100	2745.0	0.002232
475.8	0.041537	1647.0	0.040448	2781.6	0.001530
512.4	0.044108	1683.6	0.041616	2818.2	0.000426
585.6	0.043871	1720.2	0.039368	2854.8	0.003817
622.2	0.040417	1756.3	0.039691	2891.4	0.002516
658.8	0.038950	1793.4	0.028603	2928.0	0.005300
695.4	0.040504	1830.0	0.003533	2964.6	0.003367
732.0	0.042822	1866.6	0.003123	3001.2	0.005481
768.6	0.041237	1903.2	-0.000513	3037.8	0.002760
805.2	0.042586	1939.8	0.004763	3074.4	0.002382
841.8	0.038808	1976.4	0.003438	3111.0	0.003793
878.4	0.040717	2013.0	0.000418	3147.6	0.003218
915.0	0.039360	2049.6	0.005284	3184.2	0.007634
951.6	0.041860	2086.2	0.001577	3220.8	0.001751
988.2	0.040299	2122.8	0.002437	3257.4	0.008848
1024.8	0.038863	2159.4	0.007184	3294.0	0.006617
1061.4	0.041844	2196.0	0.004290	3330.6	0.006120
1098.0	0.039889	2232.6	-0.000379	3367.2	0.005307
1134.6	0.039896	2269.2	0.003020	3403.8	0.004866

Experiment #26--continued

Uncolonized IRE--Ciprofloxacin 250 $\mu\text{g ml}^{-1}$

Table 30: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.002161	4062.6	0.004093	4684.8	0.001561
3477.0	0.000883	4099.2	0.004235	4721.4	0.001632
3513.6	-0.001341	4135.8	0.005418	4758.0	0.003273
3550.2	0.001908	4172.4	0.004385	4794.6	0.000797
3586.8	0.004621	4209.0	0.004960	4831.2	0.002942
3623.4	0.002453	4245.6	0.001806	4867.8	0.001262
3660.0	0.003525	4282.2	0.002776	4904.4	0.001404
3696.6	0.002066	4318.8	0.004337	4941.0	0.004795
3733.2	0.001364	4355.4	0.002626	4977.6	0.001948
3769.8	0.000457	4392.0	0.002453	5014.2	0.002114
3806.4	0.000457	4428.6	0.002043	5050.8	0.002713
3843.0	0.001159	4465.2	0.002926	5087.4	0.002634
3879.6	0.003336	4501.8	0.002894	5124.0	0.005686
3916.2	0.003951	4538.4	0.002918	5160.6	0.004322
3952.8	0.001222	4575.0	0.002287	5197.2	0.002303
3989.4	0.005481	4611.6	0.002729		
4026.0	0.000371	4648.2	0.000765		

Experiment #27

Uncolonized IRE--Levofloxacin 500 $\mu\text{g ml}^{-1}$ **Table 31: FTIR delivery data for uncolonized IRE experiment #27, levofloxacin 500 $\mu\text{g ml}^{-1}$, using band 995-965 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.045205	2305.8	-0.007623
36.6	0.004493	1207.8	0.059533	2342.4	-0.009406
73.2	0.060608	1244.4	0.043876	2379.0	-0.005097
109.8	0.044069	1281.0	0.053195	2415.6	-0.007929
146.4	0.064236	1317.6	0.066850	2452.2	-0.003077
183.0	0.065163	1354.2	0.061089	2488.8	0.009127
219.6	0.052793	1390.8	0.046551	2525.4	-0.010036
256.2	0.034216	1427.4	0.062444	2562.0	-0.010394
292.4	0.065347	1464.0	0.048518	2598.6	0.005070
329.4	0.041280	1500.6	0.059935	2635.2	0.000717
366.0	0.056395	1537.2	0.054270	2671.8	-0.003471
402.6	0.044637	1573.8	0.063869	2708.4	0.007912
439.2	0.069613	1610.4	0.054192	2745.0	-0.012326
475.8	0.064464	1647.0	0.044130	2781.6	0.005980
512.4	0.053204	1683.6	0.045703	2818.2	-0.003200
585.6	0.049655	1720.2	0.049471	2854.8	-0.012956
622.2	0.071571	1756.3	0.045004	2891.4	-0.003978
658.8	0.046857	1793.4	0.003156	2928.0	0.010552
695.4	0.047548	1830.0	0.002535	2964.6	-0.012589
732.0	0.051080	1866.6	0.003427	3001.2	-0.009511
768.6	0.062331	1903.2	0.007973	3037.8	-0.012693
805.2	0.051132	1939.8	0.006784	3074.4	0.011802
841.8	0.053492	1976.4	-0.003812	3111.0	-0.002247
878.4	0.066640	2013.0	0.009695	3147.6	-0.005744
915.0	0.046359	2049.6	0.007833	3184.2	-0.007553
951.6	0.059577	2086.2	0.001399	3220.8	-0.002290
988.2	0.064647	2122.8	0.006557	3257.4	-0.009765
1024.8	0.053877	2159.4	-0.006906	3294.0	-0.008436
1061.4	0.048387	2196.0	-0.006696	3330.6	-0.006661
1098.0	0.052907	2232.6	0.006032	3367.2	-0.012615
1134.6	0.050800	2269.2	-0.012405	3403.8	-0.010622

Experiment #27--continued

Uncolonized IRE--Levofloxacin 500 $\mu\text{g ml}^{-1}$

Table 31: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	-0.005202	4062.6	-0.002474	4684.8	-0.009966
3477.0	-0.012501	4099.2	-0.007107	4721.4	-0.002019
3513.6	-0.010176	4135.8	0.009642	4758.0	0.002028
3550.2	0.007824	4172.4	-0.003156	4794.6	-0.000332
3586.8	0.000525	4209.0	-0.005079	4831.2	0.002168
3623.4	-0.018367	4245.6	-0.002334	4867.8	-0.006897
3660.0	-0.000918	4282.2	-0.004729	4904.4	-0.000638
3696.6	-0.013716	4318.8	0.005394	4941.0	0.000288
3733.2	0.001949	4355.4	-0.004520	4977.6	-0.002902
3769.8	-0.016112	4392.0	0.001329	5014.2	-0.016522
3806.4	0.002701	4428.6	-0.008567	5050.8	-0.005630
3843.0	0.008663	4465.2	-0.007404	5087.4	-0.004275
3879.6	-0.011915	4501.8	0.001171	5124.0	-0.017021
3916.2	-0.004240	4538.4	0.004747	5160.6	-0.008952
3952.8	0.006801	4575.0	-0.001451	5197.2	-0.005280
3989.4	-0.010901	4611.6	-0.015692		
4026.0	0.008777	4648.2	0.002736		

Experiment #27

Uncolonized IRE--Ciprofloxacin 500 $\mu\text{g ml}^{-1}$

Table 32: FTIR delivery data for uncolonized IRE experiment #27, ciprofloxacin 500 $\mu\text{g ml}^{-1}$, using band 1350-1325 cm^{-1} .

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.056259	2305.8	0.008008
36.6	0.021210	1207.8	0.059152	2342.4	0.005683
73.2	0.054431	1244.4	0.055014	2379.0	0.004942
109.8	0.057055	1281.0	0.058482	2415.6	0.005580
146.4	0.056653	1317.6	0.058640	2452.2	0.006534
183.0	0.059680	1354.2	0.057497	2488.8	0.004406
219.6	0.057662	1390.8	0.058206	2525.4	0.004981
256.2	0.061469	1427.4	0.056125	2562.0	0.005754
292.4	0.058009	1464.0	0.054257	2598.6	0.002703
329.4	0.056795	1500.6	0.057071	2635.2	0.006266
366.0	0.058112	1537.2	0.059712	2671.8	0.005344
402.6	0.057505	1573.8	0.056449	2708.4	0.003263
439.2	0.061619	1610.4	0.056196	2745.0	0.009726
475.8	0.056906	1647.0	0.054990	2781.6	0.006227
512.4	0.058947	1683.6	0.057583	2818.2	0.004319
585.6	0.057371	1720.2	0.055818	2854.8	0.003231
622.2	0.058711	1756.3	0.058955	2891.4	0.004989
658.8	0.061028	1793.4	0.043050	2928.0	0.004753
695.4	0.058175	1830.0	0.007874	2964.6	0.004737
732.0	0.057213	1866.6	0.002766	3001.2	0.005454
768.6	0.054730	1903.2	0.003302	3037.8	0.005391
805.2	0.059254	1939.8	0.007346	3074.4	0.006597
841.8	0.056701	1976.4	0.005651	3111.0	0.001750
878.4	0.053919	2013.0	0.004871	3147.6	0.007157
915.0	0.059790	2049.6	0.007866	3184.2	0.005691
951.6	0.058482	2086.2	0.009174	3220.8	0.002443
988.2	0.057386	2122.8	0.003878	3257.4	0.004390
1024.8	0.056472	2159.4	0.004973	3294.0	0.009261
1061.4	0.058017	2196.0	0.006006	3330.6	0.003988
1098.0	0.056338	2232.6	0.003539	3367.2	0.006479
1134.6	0.056685	2269.2	0.006889	3403.8	0.006069

Experiment #27--continued

Uncolonized IRE--Ciprofloxacin 500 $\mu\text{g ml}^{-1}$

Table 32: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.007700	4062.6	0.002522	4684.8	0.006621
3477.0	0.004035	4099.2	0.002719	4721.4	0.007109
3513.6	0.005068	4135.8	0.007030	4758.0	0.004256
3550.2	0.004926	4172.4	0.004768	4794.6	0.003050
3586.8	0.006833	4209.0	0.008079	4831.2	0.004335
3623.4	0.003925	4245.6	0.005722	4867.8	0.005533
3660.0	0.009048	4282.2	0.007180	4904.4	0.005044
3696.6	0.004075	4318.8	0.006227	4941.0	0.003941
3733.2	0.003570	4355.4	0.006227	4977.6	0.005848
3769.8	0.004556	4392.0	0.001143	5014.2	0.009064
3806.4	0.002333	4428.6	0.007220	5050.8	0.005541
3843.0	0.007748	4465.2	0.004910	5087.4	0.008512
3879.6	0.003775	4501.8	0.006550	5124.0	0.006376
3916.2	0.002987	4538.4	0.004217	5160.6	0.003626
3952.8	0.004895	4575.0	0.005895	5197.2	0.003933
3989.4	0.005832	4611.6	0.004256		
4026.0	0.004627	4648.2	0.006439		

Experiment #28

Uncolonized IRE--Levofloxacin 100 $\mu\text{g ml}^{-1}$ **Table 33: FTIR delivery data for uncolonized IRE experiment #28, levofloxacin 100 $\mu\text{g ml}^{-1}$, using band 995-965 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.017382	2305.8	0.004466
36.6	0.020064	1207.8	0.021788	2342.4	0.016468
73.2	0.028583	1244.4	0.027608	2379.0	-0.007700
109.8	0.006544	1281.0	0.023840	2415.6	0.020150
146.4	0.010881	1317.6	0.019546	2452.2	0.017615
183.0	0.027246	1354.2	0.016158	2488.8	0.010674
219.6	0.012450	1390.8	0.011364	2525.4	0.009933
256.2	0.028341	1427.4	0.031074	2562.0	0.012666
292.4	0.022883	1464.0	0.018020	2598.6	-0.002078
329.4	0.013287	1500.6	0.011881	2635.2	0.009950
366.0	0.019262	1537.2	0.016123	2671.8	0.002311
402.6	0.018055	1573.8	0.025444	2708.4	-0.000034
439.2	0.009778	1610.4	0.022823	2745.0	0.006570
475.8	0.024108	1647.0	0.020659	2781.6	-0.001147
512.4	0.030773	1683.6	0.017339	2818.2	0.015028
585.6	0.009243	1720.2	0.022702	2854.8	0.004820
622.2	0.021633	1756.3	0.007674	2891.4	0.008424
658.8	0.033842	1793.4	0.014356	2928.0	0.006829
695.4	0.023745	1830.0	0.005044	2964.6	-0.000836
732.0	0.027384	1866.6	0.022547	3001.2	-0.006630
768.6	0.015994	1903.2	0.015304	3037.8	-0.005880
805.2	0.032057	1939.8	0.016736	3074.4	0.017055
841.8	0.034247	1976.4	0.015175	3111.0	0.016348
878.4	0.014839	2013.0	0.002138	3147.6	-0.000155
915.0	0.030531	2049.6	0.014382	3184.2	0.020788
951.6	0.017244	2086.2	0.007976	3220.8	0.014511
988.2	0.005656	2122.8	-0.009864	3257.4	0.016442
1024.8	0.007027	2159.4	0.019357	3294.0	0.013847
1061.4	0.026884	2196.0	0.006518	3330.6	0.004458
1098.0	0.036912	2232.6	0.019546	3367.2	0.011545
1134.6	0.034799	2269.2	-0.002699	3403.8	0.013468

Experiment #28--continued

Uncolonized IRE--Levofloxacin 100 $\mu\text{g ml}^{-1}$

Table 33: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.011450	4062.6	0.008588	4684.8	0.004070
3477.0	0.006751	4099.2	0.014175	4721.4	0.014589
3513.6	0.016313	4135.8	-0.000526	4758.0	0.000819
3550.2	0.002397	4172.4	0.009786	4794.6	0.000259
3586.8	0.013123	4209.0	0.012442	4831.2	0.014020
3623.4	0.008976	4245.6	0.014571	4867.8	0.002345
3660.0	0.007519	4282.2	0.019650	4904.4	0.000009
3696.6	0.004371	4318.8	0.005346	4941.0	0.011286
3733.2	0.009700	4355.4	-0.000897	4977.6	-0.004785
3769.8	0.002923	4392.0	0.007889	5014.2	0.008941
3806.4	0.001940	4428.6	-0.002121	5050.8	0.013968
3843.0	0.001940	4465.2	-0.002828	5087.4	0.003035
3879.6	0.003656	4501.8	0.005027	5124.0	0.005458
3916.2	0.007131	4538.4	0.009484	5160.6	0.010959
3952.8	0.017374	4575.0	0.011390		
3989.4	0.016201	4611.6	0.006639		
4026.0	0.001276	4648.2	0.006070		

Experiment #28

Uncolonized IRE--Ciprofloxacin 100 $\mu\text{g ml}^{-1}$ **Table 34: FTIR delivery data for uncolonized IRE experiment #28, ciprofloxacin 100 $\mu\text{g ml}^{-1}$, using band 1350-1325 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.028853	2305.8	0.006680
36.6	0.003111	1207.8	0.026002	2342.4	0.004545
73.2	0.035328	1244.4	0.028404	2379.0	0.008830
109.8	0.033879	1281.0	0.027081	2415.6	0.006538
146.4	0.036179	1317.6	0.025868	2452.2	0.005656
183.0	0.034406	1354.2	0.029617	2488.8	0.003655
219.6	0.031382	1390.8	0.027034	2525.4	0.009447
256.2	0.029507	1427.4	0.028207	2562.0	0.005837
292.4	0.030436	1464.0	0.025970	2598.6	0.003710
329.4	0.030350	1500.6	0.024930	2635.2	0.005616
366.0	0.026372	1537.2	0.028333	2671.8	0.007247
402.6	0.026325	1573.8	0.026317	2708.4	0.006042
439.2	0.029767	1610.4	0.026396	2745.0	0.001764
475.8	0.028467	1647.0	0.024458	2781.6	0.003852
512.4	0.031074	1683.6	0.026939	2818.2	0.005703
585.6	0.028562	1720.2	0.026813	2854.8	0.004616
622.2	0.028711	1756.3	0.025340	2891.4	0.004206
658.8	0.026703	1793.4	0.013131	2928.0	0.003915
695.4	0.027640	1830.0	0.001560	2964.6	0.004222
732.0	0.026734	1866.6	0.001166	3001.2	0.004175
768.6	0.027672	1903.2	-0.000670	3037.8	0.004135
805.2	0.027932	1939.8	0.000575	3074.4	0.004135
841.8	0.025537	1976.4	-0.000725	3111.0	0.001245
878.4	0.028688	2013.0	0.003474	3147.6	0.007066
915.0	0.027616	2049.6	0.006806	3184.2	0.003332
951.6	0.023670	2086.2	0.006018	3220.8	0.004726
988.2	0.026238	2122.8	0.004970	3257.4	0.000874
1024.8	0.028514	2159.4	0.002938	3294.0	0.006372
1061.4	0.026025	2196.0	0.003222	3330.6	0.003852
1098.0	0.027435	2232.6	0.006183	3367.2	0.006955
1134.6	0.027097	2269.2	0.004324	3403.8	0.000803

Experiment #28--continued

Uncolonized IRE--Ciprofloxacin 100 $\mu\text{g ml}^{-1}$

Table 34: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.003852	4062.6	0.004191	4684.8	0.000896
3477.0	0.006821	4099.2	0.007113	4721.4	0.000823
3513.6	0.002592	4135.8	0.003371	4758.0	0.006010
3550.2	0.003190	4172.4	0.003749	4794.6	0.005262
3586.8	0.006514	4209.0	0.002190	4831.2	0.005010
3623.4	0.005813	4245.6	0.003174	4867.8	0.006175
3660.0	0.005364	4282.2	0.006947	4904.4	0.006270
3696.6	0.003852	4318.8	0.004261	4941.0	0.003734
3733.2	0.005687	4355.4	0.005159	4977.6	0.003742
3769.8	0.004293	4392.0	0.006435	5014.2	0.005537
3806.4	0.007129	4428.6	0.003167	5050.8	0.005758
3843.0	0.006687	4465.2	0.006632	5087.4	0.003119
3879.6	0.004679	4501.8	0.004939	5124.0	0.002402
3916.2	0.007373	4538.4	0.005025	5160.6	0.006617
3952.8	0.005396	4575.0	0.004356		
3989.4	0.004592	4611.6	0.001116		
4026.0	0.005561	4648.2	0.006357		

FTIR Delivery Data—Colonized Experiments

Experiment #15

Colonized IRE--Levofloxacin 500 $\mu\text{g ml}^{-1}$ **Table 35: FTIR delivery data for colonized IRE experiment #15, levofloxacin 500 $\mu\text{g ml}^{-1}$, using band 995-965 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1061.4	0.086349	2086.2	0.041943
36.6	0.030646	1098.0	0.093637	2122.8	0.039831
73.2	0.055505	1134.6	0.090348	2159.4	0.039112
109.8	0.059117	1171.2	0.092172	2196.0	0.038456
146.4	0.066460	1207.8	0.092918	2232.6	0.035409
183.0	0.072436	1244.4	0.093808	2269.2	0.034627
219.6	0.068716	1281.0	0.095947	2305.8	0.038249
256.2	0.076121	1317.6	0.096971	2342.4	0.031770
292.4	0.073425	1354.2	0.096180	2379.0	0.034735
329.4	0.084362	1390.8	0.099623	2415.6	0.036434
366.0	0.076238	1427.4	0.092586	2452.2	0.037369
402.6	0.077415	1464.0	0.093970	2488.8	0.035122
439.2	0.077020	1500.6	0.095183	2525.4	0.035724
475.8	0.082610	1537.2	0.099389	2562.0	0.030853
512.4	0.080696	1573.8	0.098697	2598.6	0.031671
585.6	0.080480	1610.4	0.100081	2635.2	0.033873
622.2	0.080174	1647.0	0.099101	2671.8	0.031734
658.8	0.087517	1683.6	0.098607	2708.4	0.030547
695.4	0.085486	1720.2	0.103649	2745.0	0.031302
732.0	0.085899	1756.3	0.103846	2781.6	0.029523
768.6	0.082799	1793.4	0.075887	2818.2	0.029729
805.2	0.088308	1830.0	0.058048	2854.8	0.028390
841.8	0.085225	1866.6	0.052566	2891.4	0.020149
878.4	0.090977	1903.2	0.047686	2928.0	0.029127
915.0	0.083616	1939.8	0.043830	2964.6	0.029882
951.6	0.090069	1976.4	0.044990	3001.2	0.026323
988.2	0.086024	2013.0	0.043399	3037.8	0.025254
1024.8	0.089296	2049.6	0.039211	3074.4	0.024382

Experiment #15--continued

Colonized IRE--Levofloxacin 500 $\mu\text{g ml}^{-1}$

Table 35: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3111.0	0.023996	3843.0	0.014322	4575.0	0.020804
3147.6	0.022333	3879.6	0.014434	4611.6	0.017094
3184.2	0.024229	3916.2	0.016464	4648.2	0.017780
3220.8	0.020670	3952.8	0.018970	4684.8	0.010178
3257.4	0.021812	3989.4	0.014840	4721.4	0.006314
3294.0	0.023474	4026.0	0.018732	4758.0	0.011102
3330.6	0.024400	4062.6	0.013580	4794.6	0.013678
3367.2	0.018460	4099.2	0.016660	4831.2	0.012404
3403.8	0.021578	4135.8	0.012460	4867.8	0.008974
3440.4	0.020410	4172.4	0.010752	4904.4	0.006944
3477.0	0.018729	4209.0	0.006678	4941.0	0.007742
3513.6	0.014681	4245.6	0.021504	4977.6	0.015498
3550.2	0.018690	4282.2	0.020790	5014.2	0.012180
3586.8	0.016408	4318.8	0.014000	5050.8	0.014854
3623.4	0.015946	4355.4	0.015540	5087.4	0.004074
3660.0	0.018928	4392.0	0.009282	5124.0	0.006720
3696.6	0.015008	4428.6	0.010220	5160.6	0.012964
3733.2	0.019768	4465.2	0.009128	5197.2	0.008316
3769.8	0.014742	4501.8	0.008470		
3806.4	0.016142	4538.4	0.019866		

Experiment #15

Colonized IRE--Ciprofloxacin 500 $\mu\text{g ml}^{-1}$ **Table 36: FTIR delivery data for colonized IRE experiment #15, ciprofloxacin 500 $\mu\text{g ml}^{-1}$, using band 1350-1325 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.038252	2305.8	0.008046
36.6	0.017688	1207.8	0.041063	2342.4	0.006243
73.2	0.026910	1244.4	0.040666	2379.0	0.006918
109.8	0.028339	1281.0	0.037696	2415.6	0.004122
146.4	0.029054	1317.6	0.037020	2452.2	0.003487
183.0	0.031183	1354.2	0.039983	2488.8	0.002804
219.6	0.031580	1390.8	0.039062	2525.4	0.006600
256.2	0.032827	1427.4	0.042533	2562.0	0.002113
292.4	0.034312	1464.0	0.039864	2598.6	0.004615
329.4	0.035408	1500.6	0.039149	2635.2	0.005607
366.0	0.036798	1537.2	0.040475	2671.8	0.004353
402.6	0.037060	1573.8	0.039300	2708.4	0.007283
439.2	0.034320	1610.4	0.040182	2745.0	0.006124
475.8	0.037513	1647.0	0.036774	2781.6	-0.000064
512.4	0.036512	1683.6	0.038347	2818.2	0.005893
585.6	0.034789	1720.2	0.039673	2854.8	0.008292
622.2	0.037259	1756.3	0.043247	2891.4	0.009992
658.8	0.038085	1793.4	0.034598	2928.0	0.004472
695.4	0.036671	1830.0	0.019396	2964.6	0.007625
732.0	0.035964	1866.6	0.009420	3001.2	0.007109
768.6	0.036488	1903.2	0.009976	3037.8	0.003471
805.2	0.036742	1939.8	0.011191	3074.4	0.007728
841.8	0.036266	1976.4	0.004885	3111.0	0.006394
878.4	0.036472	2013.0	0.009761	3147.6	0.004106
915.0	0.039300	2049.6	0.004051	3184.2	0.008697
951.6	0.038641	2086.2	0.005878	3220.8	0.004281
988.2	0.038728	2122.8	0.005957	3257.4	0.005917
1024.8	0.041309	2159.4	0.005139	3294.0	0.005631
1061.4	0.038855	2196.0	0.007355	3330.6	0.008594
1098.0	0.041063	2232.6	0.005194	3367.2	0.005409
1134.6	0.039769	2269.2	0.005314	3403.8	0.003717

Experiment #15--continued

Colonized IRE--Ciprofloxacin 500 $\mu\text{g ml}^{-1}$

Table 36: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.003820	4062.6	0.006588	4684.8	0.002922
3477.0	0.004869	4099.2	0.006567	4721.4	0.007971
3513.6	0.003624	4135.8	0.007785	4758.0	0.008229
3550.2	0.007651	4172.4	0.002778	4794.6	0.003717
3586.8	0.004667	4209.0	0.005421	4831.2	0.005534
3623.4	0.005885	4245.6	0.003779	4867.8	0.002241
3660.0	0.005307	4282.2	0.006515	4904.4	0.008487
3696.6	0.004564	4318.8	0.006691	4941.0	0.006350
3733.2	0.007682	4355.4	0.008322	4977.6	0.004925
3769.8	0.009210	4392.0	0.002932	5014.2	0.008622
3806.4	0.002994	4428.6	0.004626	5050.8	0.004812
3843.0	0.004533	4465.2	0.007176	5087.4	0.005235
3879.6	0.004110	4501.8	0.006195	5124.0	0.005235
3916.2	0.006619	4538.4	0.006939	5160.6	0.003645
3952.8	0.006216	4575.0	0.005390	5197.2	0.007610
3989.4	0.007661	4611.6	0.007083		
4026.0	0.004419	4648.2	0.007599		

Experiment #16

Colonized IRE--Levofloxacin 100 $\mu\text{g ml}^{-1}$ Table 37: FTIR delivery data for colonized IRE experiment #16, levofloxacin 100 $\mu\text{g ml}^{-1}$, using band 995-965 cm^{-1} .

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.015211	2305.8	0.008476
36.6	0.007238	1207.8	0.017613	2342.4	0.012033
73.2	0.012438	1244.4	0.024776	2379.0	0.014567
109.8	0.013346	1281.0	0.025511	2415.6	0.002864
146.4	0.013750	1317.6	0.022234	2452.2	0.011150
183.0	0.014757	1354.2	0.022548	2488.8	0.012916
219.6	0.017282	1390.8	0.019082	2525.4	0.015029
256.2	0.016862	1427.4	0.024223	2562.0	0.010870
292.4	0.019750	1464.0	0.021079	2598.6	0.011348
329.4	0.014452	1500.6	0.019288	2635.2	0.010597
366.0	0.017769	1537.2	0.023992	2671.8	0.007923
402.6	0.019057	1573.8	0.021071	2708.4	0.009656
439.2	0.021079	1610.4	0.023217	2745.0	0.008839
475.8	0.019148	1647.0	0.018867	2781.6	0.005365
512.4	0.018000	1683.6	0.016994	2818.2	0.006892
585.6	0.018281	1720.2	0.022036	2854.8	0.013354
622.2	0.018067	1756.3	0.022499	2891.4	0.009590
658.8	0.024091	1793.4	0.008344	2928.0	0.007098
695.4	0.022177	1830.0	0.002096	2964.6	0.011695
732.0	0.020138	1866.6	0.012058	3001.2	0.007032
768.6	0.019841	1903.2	0.008716	3037.8	0.012809
805.2	0.017868	1939.8	0.009227	3074.4	0.008418
841.8	0.019329	1976.4	0.015706	3111.0	0.011357
878.4	0.015615	2013.0	0.005703	3147.6	0.009615
915.0	0.022589	2049.6	0.006479	3184.2	0.008328
951.6	0.016977	2086.2	0.014113	3220.8	0.009095
988.2	0.022540	2122.8	0.005992	3257.4	0.006363
1024.8	0.019049	2159.4	0.012273	3294.0	0.001221
1061.4	0.022672	2196.0	0.005150	3330.6	0.005728
1098.0	0.019610	2232.6	0.015005	3367.2	0.008922
1134.6	0.017877	2269.2	0.006297	3403.8	0.008732

Experiment #16--continued

Colonized IRE--Levofloxacin 100 $\mu\text{g ml}^{-1}$

Table 37: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.009582	4062.6	0.010469	4684.8	0.010175
3477.0	0.013882	4099.2	0.012673	4721.4	0.006118
3513.6	0.007259	4135.8	0.002492	4758.0	0.011144
3550.2	0.005824	4172.4	0.009701	4794.6	0.007324
3586.8	0.010491	4209.0	0.011237	4831.2	0.009880
3623.4	0.007582	4245.6	0.013075	4867.8	0.008365
3660.0	0.008028	4282.2	0.009457	4904.4	0.007245
3696.6	0.013664	4318.8	0.009873	4941.0	0.007913
3733.2	0.004962	4355.4	0.009220	4977.6	0.005536
3769.8	0.005637	4392.0	0.006893	5014.2	0.003063
3806.4	0.012278	4428.6	0.008401	5050.8	0.004983
3843.0	0.005213	4465.2	0.007690	5087.4	0.007762
3879.6	0.010677	4501.8	0.004667	5124.0	0.007597
3916.2	0.004481	4538.4	0.005996	5160.6	0.008336
3952.8	0.013054	4575.0	0.011618	5197.2	0.012077
3989.4	0.005479	4611.6	0.008344		
4026.0	0.007374	4648.2	0.006175		

Experiment #16

Colonized IRE--Ciprofloxacin 100 $\mu\text{g ml}^{-1}$ **Table 38: FTIR delivery data for colonized IRE experiment #16, ciprofloxacin 100 $\mu\text{g ml}^{-1}$, using band 1350-1325 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.014493	2305.8	0.006492
36.6	0.006404	1207.8	0.015576	2342.4	0.003507
73.2	0.006701	1244.4	0.017871	2379.0	0.006797
109.8	0.009911	1281.0	0.015287	2415.6	0.004919
146.4	0.013690	1317.6	0.016347	2452.2	0.007206
183.0	0.011925	1354.2	0.009670	2488.8	0.005224
219.6	0.014027	1390.8	0.011748	2525.4	0.004630
256.2	0.014453	1427.4	0.016026	2562.0	0.010360
292.4	0.012487	1464.0	0.012719	2598.6	0.004727
329.4	0.015616	1500.6	0.014100	2635.2	0.006075
366.0	0.013923	1537.2	0.015432	2671.8	0.005361
402.6	0.010657	1573.8	0.013145	2708.4	0.006693
439.2	0.014621	1610.4	0.013482	2745.0	0.002921
475.8	0.013048	1647.0	0.010488	2781.6	0.003138
512.4	0.013329	1683.6	0.014942	2818.2	0.005232
585.6	0.014348	1720.2	0.014886	2854.8	0.006572
622.2	0.013787	1756.3	0.017013	2891.4	0.004719
658.8	0.016098	1793.4	0.011267	2928.0	0.006356
695.4	0.015167	1830.0	0.008105	2964.6	0.005224
732.0	0.015913	1866.6	0.007945	3001.2	0.005593
768.6	0.012727	1903.2	0.007174	3037.8	0.005337
805.2	0.009309	1939.8	0.005674	3074.4	0.007190
841.8	0.013153	1976.4	0.006516	3111.0	0.003523
878.4	0.016507	2013.0	0.005593	3147.6	0.005842
915.0	0.018032	2049.6	0.005216	3184.2	0.008522
951.6	0.013787	2086.2	0.005248	3220.8	0.005754
988.2	0.014003	2122.8	0.003041	3257.4	0.004012
1024.8	0.011861	2159.4	0.006556	3294.0	0.003499
1061.4	0.015127	2196.0	0.007038	3330.6	0.004590
1098.0	0.012759	2232.6	0.004414	3367.2	0.004558
1134.6	0.016483	2269.2	0.007463	3403.8	0.005264

Experiment #16--continued

Colonized IRE--Ciprofloxacin 100 $\mu\text{g ml}^{-1}$

Table 38: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.003290	4062.6	0.006457	4684.8	0.005314
3477.0	0.007086	4099.2	0.004673	4721.4	0.005070
3513.6	0.005800	4135.8	0.006846	4758.0	0.006831
3550.2	0.007215	4172.4	0.004963	4794.6	0.003347
3586.8	0.006815	4209.0	0.004399	4831.2	0.005550
3623.4	0.005099	4245.6	0.003943	4867.8	0.004096
3660.0	0.006190	4282.2	0.004490	4904.4	0.005466
3696.6	0.005314	4318.8	0.005771	4941.0	0.003812
3733.2	0.004808	4355.4	0.005093	4977.6	0.003324
3769.8	0.004036	4392.0	0.006358	5014.2	0.004361
3806.4	0.002889	4428.6	0.002775	5050.8	0.004330
3843.0	0.006228	4465.2	0.002981	5087.4	0.005001
3879.6	0.006686	4501.8	0.004971	5124.0	0.003126
3916.2	0.004193	4538.4	0.006244	5160.6	0.006732
3952.8	0.007090	4575.0	0.004483		
3989.4	0.006167	4611.6	0.006038		
4026.0	0.003332	4648.2	0.004963		

Experiment #17

Colonized IRE--Levofloxacin 250 $\mu\text{g ml}^{-1}$ **Table 39: FTIR delivery data for colonized IRE experiment #17, levofloxacin 250 $\mu\text{g ml}^{-1}$, using band 995-965 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.115514	2305.8	0.024392
36.6	0.009576	1207.8	0.116904	2342.4	0.023225
73.2	0.044514	1244.4	0.115108	2379.0	0.020527
109.8	0.059528	1281.0	0.114289	2415.6	0.026602
146.4	0.066895	1317.6	0.117269	2452.2	0.020717
183.0	0.077862	1354.2	0.113916	2488.8	0.019393
219.6	0.080568	1390.8	0.119007	2525.4	0.019136
256.2	0.084492	1427.4	0.113403	2562.0	0.016405
292.4	0.093240	1464.0	0.116598	2598.6	0.018996
329.4	0.091767	1500.6	0.119363	2635.2	0.015288
366.0	0.098819	1537.2	0.115282	2671.8	0.014452
402.6	0.098314	1573.8	0.115141	2708.4	0.011563
439.2	0.098637	1610.4	0.116441	2745.0	0.014990
475.8	0.099531	1647.0	0.119917	2781.6	0.006655
512.4	0.099779	1683.6	0.120927	2818.2	0.008782
585.6	0.103578	1720.2	0.123509	2854.8	0.011820
622.2	0.104944	1756.3	0.121730	2891.4	0.010578
658.8	0.107071	1793.4	0.118998	2928.0	0.008120
695.4	0.105714	1830.0	0.099225	2964.6	0.005711
732.0	0.107990	1866.6	0.081702	3001.2	0.008534
768.6	0.109223	1903.2	0.068476	3037.8	0.006423
805.2	0.105283	1939.8	0.059777	3074.4	0.004420
841.8	0.106235	1976.4	0.054537	3111.0	0.007714
878.4	0.105706	2013.0	0.053726	3147.6	0.006299
915.0	0.107899	2049.6	0.043181	3184.2	0.009411
951.6	0.111533	2086.2	0.040375	3220.8	0.005132
988.2	0.114728	2122.8	0.039647	3257.4	0.002301
1024.8	0.112642	2159.4	0.033116	3294.0	0.002193
1061.4	0.116540	2196.0	0.032984	3330.6	0.008459
1098.0	0.112369	2232.6	0.027629	3367.2	0.004503
1134.6	0.114504	2269.2	0.024401	3403.8	0.001465

Experiment #17--continued

Colonized IRE--Levofloxacin 250 $\mu\text{g ml}^{-1}$

Table 39: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.001142	4062.6	0.005008	4684.8	0.000017
3477.0	0.001589	4099.2	0.000372	4721.4	0.001167
3513.6	0.005521	4135.8	0.002243	4758.0	0.000298
3550.2	0.004825	4172.4	0.000058	4794.6	0.002342
3586.8	0.000025	4209.0	0.001208	4831.2	0.001366
3623.4	0.000728	4245.6	0.001299	4867.8	0.001546
3660.0	0.000803	4282.2	0.000364	4904.4	0.002359
3696.6	0.001035	4318.8	0.001316	4941.0	0.003452
3733.2	0.004453	4355.4	0.003816	4977.6	0.001639
3769.8	0.002276	4392.0	0.002185	5014.2	0.002988
3806.4	0.003948	4428.6	0.002500	5050.8	0.004263
3843.0	0.002094	4465.2	0.011596	5087.4	0.003741
3879.6	0.001622	4501.8	0.001266	5124.0	0.002765
3916.2	0.005239	4538.4	0.001829	5160.6	0.002541
3952.8	0.004668	4575.0	0.002044	5197.2	0.002798
3989.4	0.000513	4611.6	0.001746		
4026.0	0.000513	4648.2	0.003195		

Experiment #17

Colonized IRE--Ciprofloxacin 250 $\mu\text{g ml}^{-1}$ **Table 40: FTIR delivery data for colonized IRE experiment #17, ciprofloxacin 250 $\mu\text{g ml}^{-1}$, using band 1350-1325 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.047340	2305.8	0.006440
36.6	0.003330	1207.8	0.043940	2342.4	0.009330
73.2	0.024100	1244.4	0.046270	2379.0	0.004980
109.8	0.028290	1281.0	0.047960	2415.6	0.002320
146.4	0.029040	1317.6	0.050080	2452.2	0.004780
183.0	0.035420	1354.2	0.044570	2488.8	0.002790
219.6	0.034690	1390.8	0.049630	2525.4	0.005950
256.2	0.035110	1427.4	0.047510	2562.0	0.002220
292.4	0.036910	1464.0	0.048080	2598.6	0.003600
329.4	0.039670	1500.6	0.049120	2635.2	0.003610
366.0	0.036680	1537.2	0.050110	2671.8	0.004410
402.6	0.040490	1573.8	0.049040	2708.4	0.007470
439.2	0.038590	1610.4	0.049220	2745.0	0.009320
475.8	0.041830	1647.0	0.046470	2781.6	0.008110
512.4	0.039660	1683.6	0.045050	2818.2	0.003120
585.6	0.042170	1720.2	0.045560	2854.8	0.007200
622.2	0.042820	1756.3	0.050670	2891.4	0.007080
658.8	0.037610	1793.4	0.046170	2928.0	0.002830
695.4	0.038630	1830.0	0.032300	2964.6	0.007990
732.0	0.043480	1866.6	0.018030	3001.2	0.003640
768.6	0.041450	1903.2	0.012090	3037.8	0.008550
805.2	0.044360	1939.8	0.011600	3074.4	0.005420
841.8	0.041900	1976.4	0.012430	3111.0	0.002920
878.4	0.041360	2013.0	0.008420	3147.6	0.002550
915.0	0.046910	2049.6	0.006850	3184.2	0.005840
951.6	0.045650	2086.2	0.008280	3220.8	0.005390
988.2	0.044920	2122.8	0.005610	3257.4	0.003570
1024.8	0.044940	2159.4	0.006440	3294.0	0.003690
1061.4	0.044930	2196.0	0.003740	3330.6	0.008630
1098.0	0.044250	2232.6	0.002990	3367.2	0.005710
1134.6	0.050010	2269.2	0.004880	3403.8	0.006610

Experiment #17--continued

Colonized IRE--Ciprofloxacin 250 $\mu\text{g ml}^{-1}$

Table 40: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.007290	4062.6	0.008000	4684.8	0.004330
3477.0	0.005890	4099.2	0.008000	4721.4	0.002170
3513.6	0.007690	4135.8	0.007720	4758.0	0.003590
3550.2	0.003460	4172.4	0.007720	4794.6	0.004590
3586.8	0.002060	4209.0	0.007050	4831.2	0.006950
3623.4	0.004720	4245.6	0.001800	4867.8	0.006960
3660.0	0.003220	4282.2	0.005760	4904.4	0.006540
3696.6	0.003740	4318.8	0.007840	4941.0	0.008090
3733.2	0.004270	4355.4	0.009420	4977.6	0.004810
3769.8	0.006870	4392.0	0.005570	5014.2	0.002420
3806.4	0.006870	4428.6	0.002380	5050.8	0.007320
3843.0	0.008960	4465.2	0.004220	5087.4	0.005410
3879.6	0.004600	4501.8	0.005370	5124.0	0.000840
3916.2	0.005980	4538.4	0.006360	5160.6	0.002920
3952.8	0.004200	4575.0	0.006540	5197.2	0.005430
3989.4	0.007960	4611.6	0.007110		
4026.0	0.001530	4648.2	0.006120		

Experiment #18

Colonized IRE--Levofloxacin 500 $\mu\text{g ml}^{-1}$ **Table 41: FTIR delivery data for colonized IRE experiment #18, levofloxacin 500 $\mu\text{g ml}^{-1}$, using band 995-965 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.071526	2305.8	0.020590
36.6	0.021682	1207.8	0.076558	2342.4	0.022485
73.2	0.036520	1244.4	0.073099	2379.0	0.020697
109.8	0.042413	1281.0	0.070343	2415.6	0.018645
146.4	0.044448	1317.6	0.073148	2452.2	0.019630
183.0	0.050589	1354.2	0.072370	2488.8	0.018902
219.6	0.053651	1390.8	0.076674	2525.4	0.019679
256.2	0.052079	1427.4	0.075217	2562.0	0.015252
292.4	0.050001	1464.0	0.075979	2598.6	0.018628
329.4	0.059469	1500.6	0.075217	2635.2	0.015914
366.0	0.056018	1537.2	0.075739	2671.8	0.017743
402.6	0.057152	1573.8	0.075052	2708.4	0.016808
439.2	0.061331	1610.4	0.079578	2745.0	0.016311
475.8	0.061943	1647.0	0.075813	2781.6	0.013969
512.4	0.058641	1683.6	0.077005	2818.2	0.011652
585.6	0.063946	1720.2	0.081126	2854.8	0.009004
622.2	0.062489	1756.3	0.079818	2891.4	0.015260
658.8	0.064765	1793.4	0.050175	2928.0	0.012836
695.4	0.062994	1830.0	0.033251	2964.6	0.009749
732.0	0.063482	1866.6	0.031034	3001.2	0.012819
768.6	0.064202	1903.2	0.028327	3037.8	0.011528
805.2	0.063027	1939.8	0.033533	3074.4	0.014069
841.8	0.067107	1976.4	0.029114	3111.0	0.010841
878.4	0.067231	2013.0	0.022634	3147.6	0.008756
915.0	0.064914	2049.6	0.029428	3184.2	0.011412
951.6	0.066520	2086.2	0.023048	3220.8	0.006447
988.2	0.069110	2122.8	0.023552	3257.4	0.008234
1024.8	0.065618	2159.4	0.022659	3294.0	0.014565
1061.4	0.069855	2196.0	0.027881	3330.6	0.009103
1098.0	0.068224	2232.6	0.022799	3367.2	0.007688
1134.6	0.072312	2269.2	0.018513	3403.8	0.005752

Experiment #18--continued

Colonized IRE--Levofloxacin 500 $\mu\text{g ml}^{-1}$

Table 41: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.003765	4062.6	0.005296	4684.8	0.001961
3477.0	0.007423	4099.2	0.000571	4721.4	0.002284
3513.6	0.003327	4135.8	0.001208	4758.0	0.001382
3550.2	0.007796	4172.4	0.002259	4794.6	0.001672
3586.8	0.004709	4209.0	-0.003252	4831.2	0.007696
3623.4	0.004436	4245.6	0.003931	4867.8	0.001771
3660.0	0.002127	4282.2	0.001258	4904.4	0.001192
3696.6	0.000902	4318.8	0.002110	4941.0	0.005561
3733.2	0.000141	4355.4	0.000670	4977.6	0.000794
3769.8	0.005958	4392.0	-0.001920	5014.2	0.001498
3806.4	0.002119	4428.6	0.002863	5050.8	-0.003815
3843.0	-0.002549	4465.2	0.003534	5087.4	0.001068
3879.6	0.003608	4501.8	-0.000687	5124.0	0.004353
3916.2	0.001134	4538.4	0.004510	5160.6	-0.000497
3952.8	0.005205	4575.0	0.003658	5197.2	-0.000339
3989.4	0.002723	4611.6	0.001423		
4026.0	0.006232	4648.2	-0.000389		

Experiment #18

Colonized IRE--Ciprofloxacin 500 $\mu\text{g ml}^{-1}$ **Table 42: FTIR delivery data for colonized IRE experiment #18, ciprofloxacin 500 $\mu\text{g ml}^{-1}$, using band 1350-1325 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.023941	2305.8	-0.027317
36.6	0.007377	1207.8	0.022896	2342.4	-0.022805
73.2	0.024444	1244.4	0.021620	2379.0	-0.025572
109.8	0.026732	1281.0	0.022912	2415.6	-0.023818
146.4	0.026666	1317.6	0.026666	2452.2	-0.025489
183.0	0.027300	1354.2	0.024888	2488.8	-0.022624
219.6	0.027622	1390.8	0.020994	2525.4	-0.025366
256.2	0.025843	1427.4	0.022492	2562.0	-0.024271
292.4	0.025926	1464.0	0.025950	2598.6	-0.022904
329.4	0.025926	1500.6	0.025168	2635.2	-0.025728
366.0	0.025415	1537.2	0.022706	2671.8	-0.026518
402.6	0.029103	1573.8	0.022089	2708.4	-0.025604
439.2	0.027910	1610.4	0.025407	2745.0	-0.024123
475.8	0.027457	1647.0	0.021941	2781.6	-0.023209
512.4	0.025259	1683.6	0.021883	2818.2	-0.026559
585.6	0.027128	1720.2	0.022501	2854.8	-0.025283
622.2	0.027407	1756.3	0.023818	2891.4	-0.026090
658.8	0.027440	1793.4	0.009106	2928.0	-0.022278
695.4	0.026634	1830.0	-0.006842	2964.6	-0.026913
732.0	0.027350	1866.6	-0.010867	3001.2	-0.020986
768.6	0.023768	1903.2	-0.018409	3037.8	-0.023028
805.2	0.027729	1939.8	-0.019924	3074.4	-0.026955
841.8	0.027696	1976.4	-0.016836	3111.0	-0.025160
878.4	0.029803	2013.0	-0.019479	3147.6	-0.026090
915.0	0.025703	2049.6	-0.020854	3184.2	-0.024863
951.6	0.029416	2086.2	-0.020928	3220.8	-0.022813
988.2	0.026362	2122.8	-0.021439	3257.4	-0.020829
1024.8	0.025942	2159.4	-0.022649	3294.0	-0.024427
1061.4	0.025810	2196.0	-0.023563	3330.6	-0.023357
1098.0	0.024938	2232.6	-0.017108	3367.2	-0.025530
1134.6	0.020352	2269.2	-0.024781	3403.8	-0.025967

Experiment #18--continued

Colonized IRE--Ciprofloxacin 500 $\mu\text{g ml}^{-1}$

Table 42: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	-0.024287	4062.6	-0.023011	4684.8	-0.022879
3477.0	-0.027210	4099.2	-0.027440	4721.4	-0.025316
3513.6	-0.025415	4135.8	-0.025703	4758.0	-0.024649
3550.2	-0.023694	4172.4	-0.023069	4794.6	-0.024534
3586.8	-0.022641	4209.0	-0.026881	4831.2	-0.021916
3623.4	-0.023093	4245.6	-0.027235	4867.8	-0.026691
3660.0	-0.023703	4282.2	-0.025580	4904.4	-0.026502
3696.6	-0.028041	4318.8	-0.025786	4941.0	-0.026057
3733.2	-0.024402	4355.4	-0.025687	4977.6	-0.025868
3769.8	-0.023941	4392.0	-0.029622	5014.2	-0.026164
3806.4	-0.027358	4428.6	-0.025983	5050.8	-0.028050
3843.0	-0.024979	4465.2	-0.024666	5087.4	-0.026280
3879.6	-0.026930	4501.8	-0.030766	5124.0	-0.028716
3916.2	-0.022846	4538.4	-0.022064	5160.6	-0.026634
3952.8	-0.025217	4575.0	-0.024081	5197.2	-0.024509
3989.4	-0.027803	4611.6	-0.022772		
4026.0	-0.023727	4648.2	-0.026181		

Experiment #19

Colonized IRE--Levofloxacin 100 $\mu\text{g ml}^{-1}$ **Table 43: FTIR delivery data for colonized IRE experiment #19, levofloxacin 100 $\mu\text{g ml}^{-1}$, using band 995-965 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.024256	2305.8	0.008736
36.6	0.010434	1207.8	0.024247	2342.4	0.014326
73.2	0.015332	1244.4	0.023555	2379.0	0.010767
109.8	0.008798	1281.0	0.021425	2415.6	0.015494
146.4	0.017687	1317.6	0.021398	2452.2	0.008088
183.0	0.017525	1354.2	0.027258	2488.8	0.012043
219.6	0.017390	1390.8	0.023438	2525.4	0.011261
256.2	0.016420	1427.4	0.022980	2562.0	0.011108
292.4	0.017103	1464.0	0.024265	2598.6	0.008879
329.4	0.017219	1500.6	0.021668	2635.2	0.013211
366.0	0.019790	1537.2	0.024014	2671.8	0.006803
402.6	0.014640	1573.8	0.021695	2708.4	0.009940
439.2	0.017390	1610.4	0.029927	2745.0	0.009796
475.8	0.013957	1647.0	0.023160	2781.6	0.009940
512.4	0.017795	1683.6	0.025254	2818.2	0.011027
585.6	0.013984	1720.2	0.024885	2854.8	0.009796
622.2	0.020302	1756.3	0.027555	2891.4	0.012061
658.8	0.020437	1793.4	0.026908	2928.0	0.009167
695.4	0.015620	1830.0	0.024481	2964.6	0.011656
732.0	0.019907	1866.6	0.020392	3001.2	0.007441
768.6	0.016824	1903.2	0.015835	3037.8	0.011153
805.2	0.018945	1939.8	0.015134	3074.4	0.005770
841.8	0.020859	1976.4	0.009311	3111.0	0.014991
878.4	0.017974	2013.0	0.012285	3147.6	0.014029
915.0	0.020805	2049.6	0.019215	3184.2	0.011998
951.6	0.019520	2086.2	0.010758	3220.8	0.010919
988.2	0.023519	2122.8	0.010012	3257.4	0.010919
1024.8	0.021246	2159.4	0.005608	3294.0	0.009167
1061.4	0.020320	2196.0	0.004835	3330.6	0.012995
1098.0	0.022971	2232.6	0.006129	3367.2	0.018127
1134.6	0.022162	2269.2	0.009221	3403.8	0.010892

Experiment #19--continued

Colonized IRE--Levofloxacin 100 $\mu\text{g ml}^{-1}$

Table 43: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.013040	4062.6	0.019116	4684.8	0.011818
3477.0	0.016366	4099.2	0.014245	4721.4	0.015134
3513.6	0.014730	4135.8	0.015296	4758.0	0.017848
3550.2	0.007999	4172.4	0.015242	4794.6	0.013292
3586.8	0.014811	4209.0	0.013750	4831.2	0.010012
3623.4	0.014110	4245.6	0.017911	4867.8	0.015422
3660.0	0.011989	4282.2	0.013921	4904.4	0.013463
3696.6	0.013301	4318.8	0.008367	4941.0	0.016339
3733.2	0.011683	4355.4	0.013085	4977.6	0.013292
3769.8	0.015107	4392.0	0.014496	5014.2	0.015997
3806.4	0.011629	4428.6	0.013031	5050.8	0.015611
3843.0	0.017031	4465.2	0.015377	5087.4	0.015763
3879.6	0.015485	4501.8	0.016042	5124.0	0.009832
3916.2	0.013625	4538.4	0.013283	5160.6	0.018415
3952.8	0.016491	4575.0	0.010776	5197.2	0.013193
3989.4	0.012330	4611.6	0.013022		
4026.0	0.016258	4648.2	0.019385		

Experiment #19

Colonized IRE--Ciprofloxacin 100 $\mu\text{g ml}^{-1}$ **Table 44: FTIR delivery data for colonized IRE experiment #19, ciprofloxacin 100 $\mu\text{g ml}^{-1}$, using band 1350-1325 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.014739	2305.8	0.008753
36.6	0.008816	1207.8	0.013526	2342.4	0.004179
73.2	0.011683	1244.4	0.016204	2379.0	0.006498
109.8	0.010812	1281.0	0.014856	2415.6	0.008205
146.4	0.009859	1317.6	0.013076	2452.2	0.004008
183.0	0.013660	1354.2	0.016833	2488.8	0.006300
219.6	0.010506	1390.8	0.016455	2525.4	0.009050
256.2	0.016644	1427.4	0.016554	2562.0	0.007091
292.4	0.015296	1464.0	0.014946	2598.6	0.004485
329.4	0.014541	1500.6	0.017534	2635.2	0.004485
366.0	0.014901	1537.2	0.019592	2671.8	0.003981
402.6	0.015251	1573.8	0.016330	2708.4	0.006830
439.2	0.013328	1610.4	0.017893	2745.0	0.007621
475.8	0.013687	1647.0	0.017983	2781.6	0.006273
512.4	0.013013	1683.6	0.015638	2818.2	0.008223
585.6	0.013930	1720.2	0.018720	2854.8	0.006138
622.2	0.010407	1756.3	0.014730	2891.4	0.003721
658.8	0.016527	1793.4	0.016069	2928.0	0.002696
695.4	0.016042	1830.0	0.007145	2964.6	0.008160
732.0	0.014730	1866.6	0.010856	3001.2	0.004988
768.6	0.012924	1903.2	0.007828	3037.8	0.008133
805.2	0.018567	1939.8	0.008942	3074.4	0.007199
841.8	0.016159	1976.4	0.006758	3111.0	0.009481
878.4	0.017210	2013.0	0.006740	3147.6	0.006839
915.0	0.013238	2049.6	0.006893	3184.2	0.005958
951.6	0.016932	2086.2	0.008610	3220.8	0.008619
988.2	0.018208	2122.8	0.009428	3257.4	0.007693
1024.8	0.016069	2159.4	0.006650	3294.0	0.007046
1061.4	0.017893	2196.0	0.004341	3330.6	0.006408
1098.0	0.013966	2232.6	0.007666	3367.2	0.006156
1134.6	0.014739	2269.2	0.007621	3403.8	0.004035

Experiment #19--continued

Colonized IRE--Ciprofloxacin 100 $\mu\text{g ml}^{-1}$

Table 44: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.007459	4062.6	0.005851	4684.8	0.004664
3477.0	0.010659	4099.2	0.005302	4721.4	0.006929
3513.6	0.008727	4135.8	0.005653	4758.0	0.005024
3550.2	0.009526	4172.4	0.005949	4794.6	0.005168
3586.8	0.004844	4209.0	0.008079	4831.2	0.005293
3623.4	0.006525	4245.6	0.004260	4867.8	0.006489
3660.0	0.011450	4282.2	0.007100	4904.4	0.004440
3696.6	0.004143	4318.8	0.008061	4941.0	0.007091
3733.2	0.005338	4355.4	0.008223	4977.6	0.006722
3769.8	0.008951	4392.0	0.001950	5014.2	0.006120
3806.4	0.009248	4428.6	0.006336	5050.8	0.006920
3843.0	0.005248	4465.2	0.006435	5087.4	0.004592
3879.6	0.008412	4501.8	0.006084	5124.0	0.008205
3916.2	0.005626	4538.4	0.003406	5160.6	0.007999
3952.8	0.007037	4575.0	0.005994	5197.2	0.003523
3989.4	0.006345	4611.6	0.004682		
4026.0	0.005653	4648.2	0.004098		

Experiment #20

Colonized IRE--Levofloxacin 250 $\mu\text{g ml}^{-1}$ **Table 45: FTIR delivery data for colonized IRE experiment #20, levofloxacin 250 $\mu\text{g ml}^{-1}$, using band 995-965 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.046881	2305.8	0.001724
36.6	0.000853	1207.8	0.050073	2342.4	0.002347
73.2	0.004754	1244.4	0.051045	2379.0	0.001092
109.8	0.016540	1281.0	0.049262	2415.6	0.000427
146.4	0.016250	1317.6	0.054263	2452.2	0.000597
183.0	0.019408	1354.2	0.048110	2488.8	0.003243
219.6	0.020082	1390.8	0.051267	2525.4	0.000964
256.2	0.022437	1427.4	0.049142	2562.0	0.006205
292.4	0.023880	1464.0	0.051071	2598.6	0.008714
329.4	0.027763	1500.6	0.055245	2635.2	0.015371
366.0	0.027883	1537.2	0.054391	2671.8	0.014125
402.6	0.024307	1573.8	0.059213	2708.4	0.014799
439.2	0.029504	1610.4	0.056730	2745.0	0.015635
475.8	0.030759	1647.0	0.061987	2781.6	0.019476
512.4	0.027755	1683.6	0.059503	2818.2	0.011966
585.6	0.031407	1720.2	0.061680	2854.8	0.008663
622.2	0.026893	1756.3	0.065597	2891.4	0.015482
658.8	0.027942	1793.4	0.049484	2928.0	0.015661
695.4	0.035794	1830.0	0.012059	2964.6	0.016037
732.0	0.032269	1866.6	0.014842	3001.2	0.018409
768.6	0.035342	1903.2	0.013271	3037.8	0.014680
805.2	0.037860	1939.8	0.012435	3074.4	0.019007
841.8	0.038867	1976.4	0.006631	3111.0	0.015217
878.4	0.039217	2013.0	0.002663	3147.6	0.016771
915.0	0.044192	2049.6	0.004310	3184.2	0.018827
951.6	0.046454	2086.2	0.010378	3220.8	0.018930
988.2	0.042733	2122.8	0.003072	3257.4	0.013374
1024.8	0.044909	2159.4	0.002799	3294.0	0.012409
1061.4	0.045669	2196.0	0.004788	3330.6	0.014594
1098.0	0.044935	2232.6	0.010480	3367.2	0.015857
1134.6	0.048374	2269.2	0.002740	3403.8	0.022309

Experiment #20--continued

Colonized IRE--Levofloxacin 250 $\mu\text{g ml}^{-1}$

Table 45: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.020884	4062.6	-0.022909	4684.8	-0.025492
3477.0	0.024196	4099.2	-0.025137	4721.4	-0.025369
3513.6	0.024161	4135.8	-0.025144	4758.0	-0.025898
3550.2	0.024161	4172.4	-0.024048	4794.6	-0.027951
3586.8	-0.026892	4209.0	-0.027168	4831.2	-0.028394
3623.4	-0.024469	4245.6	-0.018782	4867.8	-0.032950
3660.0	-0.020407	4282.2	-0.025470	4904.4	-0.027248
3696.6	-0.021045	4318.8	-0.021966	4941.0	-0.032877
3733.2	-0.021720	4355.4	-0.022779	4977.6	-0.031999
3769.8	-0.018738	4392.0	-0.019601	5014.2	-0.028938
3806.4	-0.023475	4428.6	-0.020515	5050.8	-0.025238
3843.0	-0.024063	4465.2	-0.021858	5087.4	-0.031107
3879.6	-0.020312	4501.8	-0.022213	5124.0	-0.027937
3916.2	-0.018818	4538.4	-0.018484	5160.6	-0.032137
3952.8	-0.016373	4575.0	-0.018063		
3989.4	-0.017113	4611.6	-0.012441		
4026.0	-0.021168	4648.2	-0.021205		

Experiment #20

Colonized IRE--Ciprofloxacin 250 $\mu\text{g ml}^{-1}$ **Table 46: FTIR delivery data for colonized IRE experiment #20, ciprofloxacin 250 $\mu\text{g ml}^{-1}$, using band 1350-1325 cm^{-1} .**

Time (sec)	Band Area (abs cm^{-1})	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
0.0	0.000000	1171.2	0.037585	2305.8	0.005861
36.6	0.012185	1207.8	0.041661	2342.4	0.007594
73.2	0.025451	1244.4	0.039756	2379.0	0.006805
109.8	0.023821	1281.0	0.041626	2415.6	0.008384
146.4	0.029553	1317.6	0.038949	2452.2	0.009276
183.0	0.028978	1354.2	0.040339	2488.8	0.008332
219.6	0.031509	1390.8	0.038306	2525.4	0.008401
256.2	0.032470	1427.4	0.041515	2562.0	0.005681
292.4	0.033200	1464.0	0.043574	2598.6	0.008907
329.4	0.033543	1500.6	0.040803	2635.2	0.011421
366.0	0.035113	1537.2	0.040030	2671.8	0.009345
402.6	0.033148	1573.8	0.038237	2708.4	0.009310
439.2	0.033758	1610.4	0.042116	2745.0	0.011610
475.8	0.038409	1647.0	0.040005	2781.6	0.013352
512.4	0.034650	1683.6	0.039378	2818.2	0.012194
585.6	0.035551	1720.2	0.041240	2854.8	0.009937
622.2	0.035457	1756.3	0.045299	2891.4	0.008727
658.8	0.037456	1793.4	0.030385	2928.0	0.011293
695.4	0.039026	1830.0	0.017677	2964.6	0.010683
732.0	0.039661	1866.6	0.015738	3001.2	0.008195
768.6	0.041189	1903.2	0.011807	3037.8	0.008530
805.2	0.039412	1939.8	0.010838	3074.4	0.009499
841.8	0.041060	1976.4	0.008881	3111.0	0.012039
878.4	0.037164	2013.0	0.009336	3147.6	0.011473
915.0	0.038657	2049.6	0.008632	3184.2	0.008255
951.6	0.039455	2086.2	0.008444	3220.8	0.009594
988.2	0.038117	2122.8	0.011910	3257.4	0.013489
1024.8	0.040185	2159.4	0.008461	3294.0	0.010323
1061.4	0.035620	2196.0	0.007912	3330.6	0.009491
1098.0	0.040348	2232.6	0.009911	3367.2	0.005037
1134.6	0.035869	2269.2	0.005689	3403.8	0.007955

Experiment #20--continued

Colonized IRE--Ciprofloxacin 250 $\mu\text{g ml}^{-1}$

Table 46: Continued.

Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.	Time --cont.	Band Area --cont.
3440.4	0.013000	4062.6	0.009081	4684.8	0.008250
3477.0	0.006487	4099.2	0.010622	4721.4	0.007141
3513.6	0.007929	4135.8	0.009244	4758.0	0.011812
3550.2	0.007929	4172.4	0.009530	4794.6	0.007272
3586.8	0.008959	4209.0	0.007378	4831.2	0.007443
3623.4	0.004467	4245.6	0.005584	4867.8	0.010092
3660.0	0.004712	4282.2	0.009008	4904.4	0.012277
3696.6	0.007467	4318.8	0.010646	4941.0	0.009619
3733.2	0.011877	4355.4	0.008535	4977.6	0.009187
3769.8	0.010663	4392.0	0.007842	5014.2	0.006986
3806.4	0.007231	4428.6	0.009587	5050.8	0.008087
3843.0	0.010051	4465.2	0.007752	5087.4	0.007280
3879.6	0.007500	4501.8	0.008983	5124.0	0.007704
3916.2	0.006130	4538.4	0.009000	5160.6	0.010785
3952.8	0.008543	4575.0	0.010801	5197.2	0.008543
3989.4	0.008951	4611.6	0.013434		
4026.0	0.009163	4648.2	0.013785		

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