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# Insight into Erythromycin Sorption: Understanding the Influential Parameters and Optimization by Quality by Design

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## Abstract

Erythromycin produced by *Saccharopolyspora erythraea*, in batch mode was recovered from growth media, using a microscale approach, by selective sorption to neutral nanostructured sorbents (XAD-16N™, XAD-18™, and XAD-1600N™).

Higher erythromycin removals (more than 80%) were achieved with XAD-16N™ and XAD-18™. Sorption equilibrium, for every solute/sorbent system tested, was obtained in less than 2h contact time. A kinetic model for the sorption of erythromycin on the neutral sorbents versus time was adjusted, yielding a $q_{max}$ of 0.40, 1.34 and 0.50 mmol/g, respectively for XAD-16N™, XAD-18™ and XAD-1600N™.

Both Freundlich and Langmuir models showed a good fit for XAD-16N™ and XAD-1600N™. A stepwise isotherm model, type IV according to BET classification, fitted the sorption onto XAD-18™.

In order to predict the nature of erythromycin sorption onto XAD-16N™, XAD-18™ and XAD-1600N™, the thermodynamic parameters $\Delta H_{ads}$, $\Delta G_{ads}$ and $\Delta S_{ads}$ were estimated. A physical sorption process was observed since $\Delta H_{ads}$ was lower than 40 kJ/mol. The erythromycin sorption optimization was accomplished following a quality by design (QbD) approach.

**Keywords:** Erythromycin; *Saccharopolyspora erythraea*; sorption; neutral microstructured materials; XAD-16N™, XAD-18™ and XAD-1600N™

**Abbreviations:** C-Initial solute concentration of solute (mol/L); $C_e$-Equilibrium liquid phase concentration (mol/L); $C_t$-Solute concentration at time t (mol/L); DOE- Design of Experiments; k-Equilibrium constant (L/mol); $k_1$-Rate constant, in the mathematical non-linear model; $k_f$-Constant in Freundlich model (the dimensions depend on the dimensions of q and Ce and on the value of n); $k_L$-Constant in Langmuir model (mmol/L); n-Parameter in Freundlich isotherm model (adimensional); q-Equilibrium concentration or loading of erythromycin on sorbent (mmolsolute/gsolid); $q_e$-Equilibrium concentration or loading of erythromycin on sorbent (mmolsolute/gsolid); $q_{max}$-Maximum capacity of the sorbent (mmolsolute/gsolid); $Q_b$D-Quality by Design; R-Gas universal constant [J/(K.mol)]; T-Absolute temperature (K); t-Time (min); T-Absolute temperature (K); V-Solution volume (L); W-Weight of sorbent (g); ΔG- Free energy of sorption (J/mol); ΔH-Enthalpy of sorption (J/mol); ΔS-Entropy of sorption (J/mol.K)

## Introduction

Erythromycin is a broad spectrum macrolide antibiotic, widely used against mycoplasma, campylobacter, legionella, streptococci, aureus, and gram-negative and gram-positive bacteria. Erythromycin has a similar activity spectrum to penicillin, being used by penicillin-sensitive people. It is specially indicated in the treatment of respiratory and skcomplaintsins. Erythromycin has received increased attention due to the recent applications of its semi-synthetically modified derivatives, azithromycin, roxithromycin, and clarithromycin, to infectious diseases [1-5].

Therefore, erythromycin is the starting material for second third-generationtion semi-synthetic derivatives. Additionally, erythromycin, a potent antibiotic long-recognized as a therapeutic option for bacterial infections, is now gaining much attention as a repurposing anti-inflammatory drug [6-8].

The new findings, the effective completion of the genome sequence for the bacterium *Saccharospolyspora erythrae*, offer a host of possibilities for the production of novel antibiotics, anti-inflammatory, immunosuppressant and anti-cancer compounds, all based on this polyketide starting material [9]. Erythromycin A is also particularly attractive because of the numerous opportunities to influence the final compound structure through manipulation of either polyketide or tailoring biosynthesis, extending the molecule application in the face of acquired pathogenic drug resistance [10,11]. Heterologous biosynthesis offers the potential for rapid overproduction of original and analog forms of clinically relevant natural compounds.

Nowadays, the industrial production of erythromycin is mainly carried out by *Saccharopolyspora erythraea*. improved the production and purity of erythromycin A by metabolic engineering of the industrial erythromycin-producing strains *Saccharopolyspora erythraea* strains ZL1004 and ZL1007, in a 50 L fermentor [12]. Furthermore, fermentation of recombinant strain ZL1004 was successfully scaled up from laboratory scale (50 L fermentor) to industrial scale (25 and 132 $m^3$), with similar levels of Er-A production and purity obtained.

Zhang et al. (2010) and Jiang et al. [13,14] produced erythromycin A using *E. coli* as a heterologous host, an alternative route to the original compound, and a platform for full manipulation of the biosynthetic pathway with the express purpose of expanding molecular diversity and antibiotic activity.

However, the production and productivity of erythromycin by fermentation technology is still facing biological and engineering limitations. One of the major obstacles is the scale-up difficulty of the overall production process. The recovery is a very important step in erythromycin production. The downstream process is mainly ascribed via conventional steps: filtration (removal of biomass), solvent extraction (isolation and purification) and subsequent crystallization [15]. The *in situ* removal of products during bioconversions is a desirable concept to attain high productivity and to reduce recovery costs [15,16]. Sorption is an effective method for separation of many diluted substances and a useful separation technique for the removal of nonpolar compounds from aqueous solutions [15].

When compared to other operations, namely distillation or solvent extraction from dilute aqueous solutions, sorption present wider acceptance for large-scale separation due to the low energy nature and environment friendly of the separation processes [17].

Another situation, in which erythromycin recovery is very important, had recently been published, reporting that the presence of low concentrations of antibiotics in wastewaters may lead to the development of antibiotic resistance in the whole envi-ronment [16,18,19]. In fact, erythromycin is an antibiotic considered as a recalcitrant pollutant because it is currently found in

treated wastewaters. In addition, traces of antibiotics are a threatening ecological issue as these compounds are biologically active even at very low concentrations (ng/L) [19]. To remove erythromycin from wastewaters recent approaches include enzymatic degradation or sorption [19].

In a sorption process, a selectively binding of a single solute from a mixture, faced two main problems: (*i*) conventionally used sorbents (e.g. activated carbon) have extremely complex surface chemistry, resulting in a wide variation in the types of binding sites available to the solutes, i.e., solutes can be indiscriminately bound to the sorbents through a wide variety of mechanistic interactions; (*ii*) hydrophobic interactions are predominant mechanisms in aqueous environments, leading to sorption due to the limited solvating ability of water.

Limitations of the number of possible interaction mechanisms allow the design more selective sorbents [17]. Nowadays, neutral structured materials became available and could be used for the antibiotic downstream process. For large scale-scale operations, the most commonly used neutral structured materials are copolymers of styrene (or ethylvinylbenzene) and divinylbenzene. In past years, these materials have been improved to offer adequate mechanical strength, high surface area for sorption, and appropriate pore sizes for rapid transport. A potential advantage of structured materials is their composition that fills the requirements of the Food and Drug Administration (FDA) and their low toxicity. In this work new neutral structured materials recently approved by FDA are used for erythromycin recovery.

Sorption studies of several compounds on neutral structured materials, such as amino acids, antibiotics, limonoids, flavonoids, and enzymes had been performed [20-23].

A quality by design (QbD) approach can be used to understand the effect of erythromycin and sorbents concentration, time, and temperature on critical quality attributes to reduce the recovery variability, control the cost, and improve the safety and quality of the product [24]. The experimental design methodology is a strategy that allows the study of different variables simultaneously, the relationship between them and their influence on different experimental responses, with a small number of experiments [25]. Therefore, mathematical models allowed the determination of the optimum level of the variables required for a given response [25].

In this work, the recovery of erythromycin through the adsorption to neutral microstructured materials, XAD-16N™ XAD-18™, and XAD-1600N™, approved by the FDA, was carried out. In order, to attain these goals erythromycin was produced from *Saccharopolyspora erythraea* by fermentation in a batch mode. Kinetic, equilibrium, and thermodynamics parameters were studied as well as adsorption optimization using a quality by design approach.

## Materials and Methods

### Materials

Erythromycin ($C_{37}H_{67}NO_{13}$) was from Calciochem®. The standard solution of erythromycin was prepared according to United States Pharmacopeia (USP).

The neutral synthetic polymers, XAD-16N™ (aliphatic, polystyrene-divinylbenzene), XAD-18™ (aromatic, polystyrene-divinylbenzene) and XAD-1600N™ (aromatic, polystyrene-divinylbenzene) were a gift from Rohm and Haas, USA.

XAD-16N™ polymeric sorbent has clearance under Food and Drug Administration (FDA), Food Additive Regulation 21CFR173.65-Divinylbenzene Copolymer.

XAD-16N™, XAD-18™ and XAD-1600N™ had the following characteristics: (*i*) average pore diameter of 150 Å; (*ii*) average particle diameter of 635±75, 425±50 and 400±50 µm; (*iii*) surface area of ≥800, ≥800 and ≥700 $m^2/g$; (*iv*) pore volume of 1.39, 1.45 and 1.52 mL/g; (*v*) bulk density (g/mL) of 0.72, 0.69 and 0.66, respectively; (*vi*) wet size 20-60 mesh.

Prior to use, the synthetic neutral polymers were washed with distilled water, dried at 40 °C and kept in a desicator until use.

All other chemicals were analytical grade and obtained from various sources.

### Analytical Methods

Erythromycin was analysed by spectrophotometry, at 280 nm, and by high-performance liquid chromatography (HPLC) (LC-6 Shimadzu) according to the method of USP [26]. The chromatograph was equipped with an isocratic pump and an ultraviolet detector (SPD-6A Shimadzu UV).

### Microorganism and Culture Conditions

*Saccharopolyspora erythraea* from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) 40517 was used for the production of erythromycin.

The strain was grown on agar plates containing the following nutrients in distilled water (g/L): glucose 4.0, yeast extract 4.0, malt extract 10, calcium carbonate 2.0, and agar 12.0; pH was adjusted to 7.2 with KOH before adding agar. Spore stocks were stored in a 20 % (v/v) glycerol solution at −80 ºC.

Microbial growth was carried out in a 3.2 L bioreactor (Infors HT) at 28 ºC, pH 6.5, at 200 rpm. The defined medium contained in g/L of distilled water: glucose 35, $K_2HPO_4$ 7, $KH_2PO_4$ 3, $NaNO_3$ 2.4, and trace elements ($FeCl_3$ 0.005, $ZnCl_2$ 0.006, $MnCl_2$ 0.001, $CoCl_2$ 0.003).

## Effect of Sorbent/Erythromycin Solution Ratio on Sorption Kinetics

The adsorption experiments were carried out in 24 micro-well plates. This micro methodology shows less variability in secondary metabolite recovery, reducing the workload and costs in the antibiotic downstream process [15]. In every experiment, the media was previously equilibrated to the desired temperature before adding the sorbent.

Different amounts of XAD-16N™, XAD-18™ and XAD-1600N™ were added to 3 mL of a model-growth media containing 2.1 mmol/L of erythromycin.

Adsorption was carried out at 200 rpm in an orbital shaker, for 90 min at 30 ºC. The efficiency of the adsorption was estimated as the ratio: mass of adsorbed erythromycin/ mass of sorbent. All the assays were carried out in triplicate.

## Effect of Contact Time on Adsorption Kinetics

Adsorption studies were conducted by adding the sorbent to an aqueous solution of erythromycin, after centrifugation of the cells of *S. erytraea*. The adsorption experiments were carried out in 24 micro-well plates. In every experiment, the media was previously equilibrated to the desired temperature before adding the sorbent.

The adsorbed amounts of erythromycin, $q$, were calculated from equation 1 [27]:

$$q = (C - C_e).\ V / W \qquad \text{(eq.1)}$$

A mass of 15 mg (dry weight) of each sorbent was added to 3 mL of a growth medium containing 2.1 mmol/L of erythromycin. Adsorption was carried out, in an orbital shaker at 200 rpm and at different temperatures (30, 40, 50 and 60 ºC). Each experiment corresponded to different adsorption times (0 to 2 hours). All the assays were carried out in triplicate. At each temperature, samples were collected every 10 minutes during the first hour and after at 90 and 120 minutes. At the end of each experiment, the sorbent was separated from the solution by paper filtration. Erythromycin was assayed in triplicate.

A mathematical model was used to simulate the uptake of erythromycin against time:

$$d\,C_t / dt = -k_1\,(1 - q / q_{max})\,.\,C_t \qquad \text{(eq. 2)}$$

This model assumes that adsorption is proportional to the solute concentration in the solution and to the fraction of the unoccupied surface [27].

The equation (2) was integrated numerically and the parameters for each set of experimental data were estimated using non-linear least-square regression analysis, by minimizing the residual-sum-of squares between the experimental data points and the estimated values by the model, using a "solver" add-in from Excel for Windows, version 8.0 SR2.

## Establishment of Isotherms

For the establishment of the isotherms $q_e$ *vs* $C_e$, experimental data points were obtained by varying erythromycin concentration from 0.3 to 2.55 mmol/L, at constant sorbent concentration in a model-growth medium. The following amount, 15 mg, of sorbents XAD-16N™, XAD-18™, and XAD-1600N™ were added to 3 mL of an aqueous solution of the model-growth medium. The adsorption reaction was carried out at 30 ºC, 200 rpm in an orbital shaker. After 90 min reaction time, the sorbent was removed from the solution by filtration. Aliquots were taken from this solution and assayed for their content in erythromycin. All the experiments were carried out in triplicate.

The fit of Langmuir and Freundlich models to experimental data was carried out using a nonlinear curve-fit program in Excel for Windows, version 8.0 SR2, by minimizing the residual-sum-of squares between the experimental data points and the estimated values by the model.

The empirical Freundlich equation is expressed by:

$$q = k_f C_e^{\,n} \qquad \text{(eq. 3)}$$

where $q$ is the equilibrium concentration or loading of adsorbate on sorbent, $C_e$ is the equilibrium concentration of the solute of the fluid phase; $k_f$ is the equilibrium constant (the dimensions depend on the value of $C_e$) and $n$ is a dimensionless

constant, both are determined experimentally and are characteristic of the adsorption system [28,29]. $k_f$ increases with the total adsorption capacity to bind the adsorbate. The *n* value may vary along the adsorption process, being related to the adsorption efficiency and to the energy of adsorption [28]; *n* values lower than 1 correspond to a favourable adsorption process, while values higher than 1 indicate unfavourable process [30, 31]. The Freunhdlich equation has been widely used since it is a mathematically simple model adequate to describe (i) non-linear adsorption in a narrow range of solute concentration and also (ii) adsorption processes on surface adsorption sites that are energetically heterogeneous. This model does not consider a limit for adsorption capacity. Theoretically, the amount of adsorbed solute may become infinite as bulk solute concentration increases [30].

The Langmuir isotherm has the following form:

$$q = q_{max}\, C_e / (k_L + C_e) \qquad \text{(eq. 4)}$$

where $k_L$ is an equilibrium constant for the adsorption process (determined experimentally) [30]. This isotherm model has a theoretical basis, considering: (i) the adsorbed molecules form a monolayer on the sorbent surface; (ii) each site for adsorption is equivalent in terms of adsorption energy; (iii) there are no interactions between adjacent adsorbed molecules [32]. This isotherm and the number of sites on the sorbent are limited [30].

For the majority of the adsorption systems, the interactions between adsorbed molecules may occur leading to other profiles of adsorption isotherms. In less common situations, concave isotherms toward the ordinate, are observed. These are unfavourable isotherms since relatively low solids loading are obtained [28].

The influence of isotherm shape has been discussed to know whether adsorption is favorable or not in terms of $R_L$, a dimensionless constant referred to as the separation factor or equilibrium parameter [32]. $R_L$ is calculated using the equation (eq. 5):

$$R_L = 1 / (1 + k_L^{-1} \cdot C_e) \qquad \text{(eq. 5)}$$

## Estimation of Thermodynamic Parameters

The adsorption enthalpy ($\Delta H_{ads}$) (eq. 6) was calculated from Clasius-Clapeyron equation [33,34]:

$$ln\ C = -\ \Delta H_{ads} / RT + constant \qquad \text{(eq. 6)}$$

where *ln C* is the ln of the equilibrium erythromycin concentration in the aqueous phase [mass (mol) solute/volume (L) solution], *R* is the gas universal constant (8.314 J / mol K) and *T* is the absolute temperature.

The adsorption free energy ($\Delta G^{o}_{ads}$) was related with the equilibrium constant for the adsorption process, the Langmuir constant ($k_L$), by the equation (eq. 7) [30]:

$$\Delta G_{ads} = -\ RT\ ln\ k_L \qquad \text{(eq. 7)}$$

The entropy ($\Delta S_{ads}$) changes were calculated using equation (eq. 8):

$$\Delta S_{ads} = (\Delta H_{ads} - \Delta G_{ads}) / T \qquad \text{(eq. 8)}$$

## Design of Experiments for Optimization of Adsorption Process of Erythromycin

An experimental design was used to optimize the influence of contact time, temperature, neutral polymer concentration and antibiotic concentration on erythromycin adsorption. The four variables were tested simultaneously with a minimum number of trials, according to an adequate experimental design (Table 1), which enables to find interactions between the variables [25]. The experimental design methodology makes use of statistical tools for selecting a minimum set of experiments adequately distributed in the experimental region (experimental matrix).

The experiments were carried out following a central composite rotatable design (CCRD). For the design setup of each variable, five coded levels were established: 0 (midpoint), -1 and +1 (factorial points), $-\sqrt{\alpha}$ and $+\sqrt{\alpha}$ (axial points) (Table 1). The standard deviation of the central points was an independent estimate of the experimental error considered constant throughout the experimental domain.

| | Experimental factors | | | |
|---|---|---|---|---|
| Coded levels | Time (min) | Temperature (°C) | Neutral microstructured materials (g/L) | Erythromycin (mM) |
| $-\sqrt{a}$ | 6.5 | 25 | 0.3 | 0.8 |
| -1 | 20.0 | 35 | 2.0 | 1.5 |
| 0 | 40.0 | 50 | 4.5 | 2.5 |
| 1 | 60.0 | 65 | 7.0 | 3.5 |
| $\sqrt{a}$ | 73.5 | 75 | 8.7 | 4.2 |

**Table 1:** Coded and decoded levels of experimental factors studied in erythromycin recovery from growth media.

The response variable was the residual erythromycin in solution (not adsorbed). The experiments were performed in random order. Triplicate experiments were carried out for the central points. The choice of experimental domains resulted from preliminary studies.

Statistica™ software (Statsoft, USA), version 7, was used to generate an outline of the experiments to perform a total of 18. With CCRD, the 5 levels for each factor were used which enabled to fit of second-order polynomials to the experimental data points and the regression coefficients obtained. The results of each CCRD were analyzed using the software Statistica™, version 7, from Statsoft, USA. Both linear and quadratic effects of the four variables under study, as well as their interactions, on residual erythromycin in solution (mmol/L), were calculated. Their significance (p values) was evaluated by analysis of variance. Three-dimensional (3D) response surface plots were also constructed using Statistica™ software for a better understanding of the effect of the four variables (contact time, temperature, neutral polymer concentration, and erythromycin concentration).

The fit of the models was evaluated by the determination coefficients ($R^2$) and adjusted $R^2$ ($R^2_{adj}$).

## Results and Discussion

Erythromycin biosynthesis was growth linked in part of the exponential phase and continued during the stationary phase, attaining at this stage the maximum concentration. The cells were grown, at 28 °C, with a specific growth rate (μ) of 0.05 $h^{-1}$ and a doubling time of 14 h. The recovery of erythromycin from growth media was carried out in batch mode by selective adsorption onto neutral polymeric materials and the results were presented and discussed.

### Kinetics

#### Effect of Sorbent Concentration

The capacity of the sorbent is often one of the most important parameters in a separation process by adsorption, as it dictates the quantity of the sorbent required. The adsorption efficiency of erythromycin onto XAD-16N™, XAD-18™ and XAD-1600N™ was evaluated in a microscale batch mode (Figure 1A). After a contact time of 90 min, removal of 80 %, 70 %, and 60 % (m/v) was achieved with low loads, less than 1 % (m/v), of XAD-16N™, XAD-18™, and XAD-1600™, respectively. The amounts of erythromycin adsorbed to the neutral microstructured sorbents tested are presented in Figure 1B. A decrease in erythromycin adsorbed per gram of sorbent was observed according to a power equation for the three sorbents tested. There is a limit for the adsorption of a solute from a solution onto a sorbent, which corresponds to the saturation. The best results were obtained with XAD-16N™ according to the following equation: $q_e = 1.39\ [Sorbent]^{-0.86}$. Total removal of erythromycin from the medium, even with low sorbent concentration, might be the reason for these results.

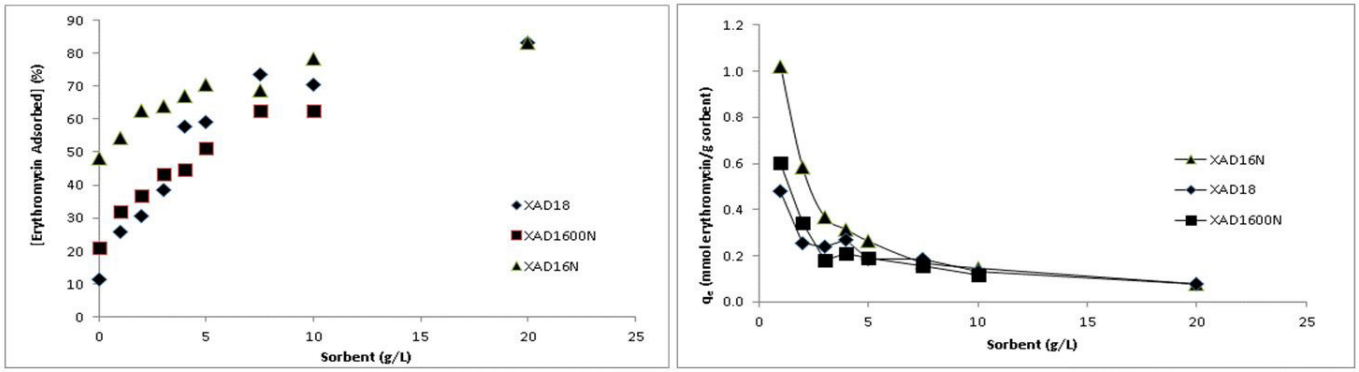


**Figure 1:** A. Efficiency of erythromycin sorption onto ▲ XAD-16N™, •XAD-18™ and ▪ XAD-1600N™ after 90 min contact time, at 30 °C; B. Evaluation of qe (mmol erythromycin/g sorbent) at different sorbent concentrations

## Effect of Contact Time and Temperature

In order to attain a high adsorption efficiency, the contact time of the sorbent and solute, and the temperature, are important parameters. An example is an application in the pharmaceutical industry that has, as one of the main goals high productivity, to minimize costs. The knowledge of the adsorption rate allows for the exact sorbent contact time, leading to maximum efficiency. The uptake of erythromycin was carried out, on a microscale, from the model-growth media, throughout the time, at different temperatures, to the neutral sorbents (XAD-16N™, XAD-18™, and XAD-1600N™) (Figure 2). Adsorption increased with temperature (30, 40, 50, and 60 ºC) and with contact time till a steady-state and quasi-equilibrium were attained. No significant variation in residual concentration of erythromycin, at different temperatures, was detected after 60 min contact with XAD-16N™, XAD-18™, and XAD-1600N™. It is further seen from Figure 2, that adsorption is fast initially, in the first 30 minutes. This can be attributed to the acceleration of some steps of the adsorption process or to the creation of new adsorption sites on the surface of the sorbent with increased temperature. These are very important results for the pharmaceutical industry since little contact time could have an impact on process economics. Also, continuous adsorption systems can be implemented.

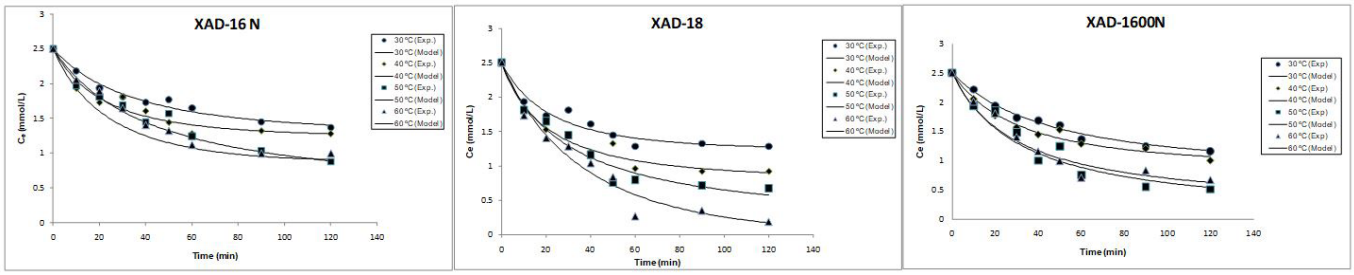


**Figure 2:** Time-course of erythromycin sorption at different temperatures, to ▲ XAD16N™, •XAD18™ and ▪ XAD1600N™; lines are models according to equation 2

| Sorbent | $k_1$ (min$^{-1}$) | | | | $q_{max}$ (mmol/g) | | | |
|---|---|---|---|---|---|---|---|---|
| | 30 ºC | 40 ºC | 50 ºC | 60 ºC | 30 ºC | 40 ºC | 50 ºC | 60 ºC |
| XAD-16N™ | 0.015 | 0.025 | 0.030 | 0.020 | 0.24 | 0.25 | 0.40 | 0.40 |
| XAD-18™ | 0.025 | 0.035 | 0.032 | 0.029 | 0.25 | 0.35 | 0.47 | 1.34 |
| XAD-1600N™ | 0.015 | 0.022 | 0.030 | 0.030 | 0.32 | 0.32 | 0.50 | 0.45 |

**Table 2:** Estimated parameters ($k_1$ and $q_{max}$) for the model fitted to the uptake of erythromycin against time at different temperatures

The model of eq. 2 can describe the adsorption kinetics for erythromycin onto XAD-16N™, XAD-18™, and XAD-1600N™ sorbents (Figure 2). The estimated parameters are presented in Table 2. According to the model, the maximum capacity ($q_{max}$) for erythromycin recovery was 0.40, 1.34, and 0.50 mmolg/g solid, respectively on XAD-16N™, XAD-18™, and XAD-1600N™ at 60 ºC and 50 ºC, respectively.

In porous materials, according to [36], an empirical relation is found between the solute adsorbed and the square root of time ($t^{1/2}$) over most of the period up to equilibrium, and in erythromycin three curve types were adjusted, which indicates the presence of diffusion limitations. Therefore in order to evaluate potential diffusional limitations, as the sorbent tested (XAD-16N™, XAD-18™, and XAD-1600N™) are porous, erythromycin adsorbed was represented graphically against the square root of time (Figure 3). A type I curve was observed for all the sorbents tested. The initial induction period was, maybe, due to a physical barrier at the polymer surface. The results indicate diffusion limitations, although the experiments were conducted with agitation (200 rpm). In addition, the maximum adsorption of erythromycin per unit surface area of sorbent was calculated, assuming monolayer adsorption. For this purpose, $q_{max}$ values, estimated from equation 2 (Table 2) were combined with the reported surface area for the polymers (*c.f.* 2.5) adjusted to a dry resin basis (*i.e.*, *ca.* 0.5 g dry resin/g wet resin). The resin XAD-18$^{TM}$ adsorbed erythromycin in a larger extent (3.35 µmol/m$^2$), than XAD-1600N$^{TM}$ (1.43 µmol/m$^2$) and XAD-16N$^{TM}$ (1.00 µmol/m$^2$).

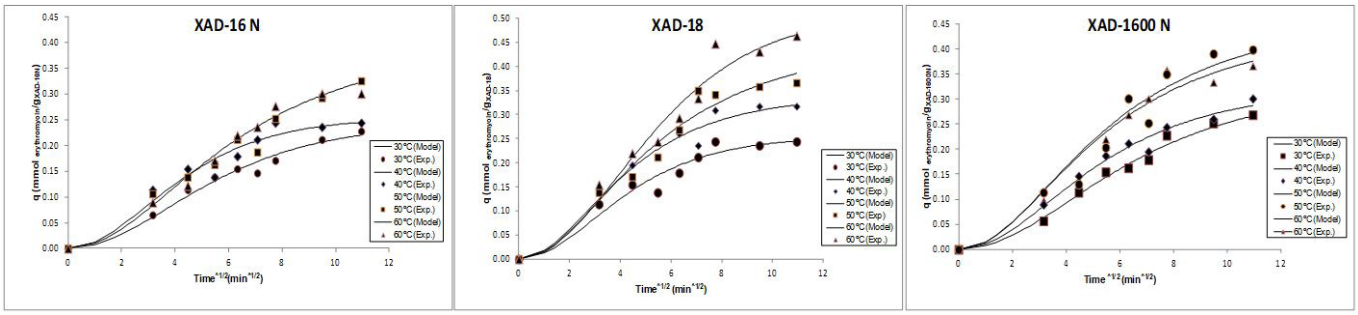


**Figure 3:** Sorption of erythromycin from production media, at different temperatures versus $t^{1/2}$ to XAD-16N™, XAD-18™ and XAD-1600N™

The affinity factors [$q_e/C_e$ (L/g)] of 0.29, 0.14 and 0.17 L/g, respectively for XAD-16N™, XAD-18™, and XAD-1600N™ demonstrated that the three microstructured materials are suitable for the adsorption of erythromycin at 30 °C.

[35] on the study about the removal of the antibiotics, penicillin, tetracycline, and cephalosporin, onto neutral polymeric sorbents (Amberlite™ XAD-4, XAD-7, and XAD-16 resins) concluded that these antibiotics adsorbed to the aromatic sorbent (Amberlite XAD-16) with a higher affinity compared to adsorption onto the aliphatic ester sorbent (XAD-7).

## Equilibrium Studies

### Adsorption Isotherms

At a given temperature, the equilibrium of adsorption is usually presented under the form of adsorption isotherms, which are useful for selecting the most appropriate sorbent and also for predicting the performance of the process [15]. Adsorption isotherms are one of the most interesting approaches, to investigating the adsorption mechanism. Mainly aspects to be considered include (*i*) the rate of adsorption, (*ii*) the shape of the isotherm, (*iii*) the significance of the plateau found in many isotherms, (*iv*) the effect of temperature, (*v*) the nature of the interaction between adsorbate and sorbent [17]. classified The isotherms into four classes according to their shape, based on the form of the initial part of the isotherm, and in subgroups related to the behavior at higher concentrations [36].

The Freundlich and Langmuir models were fitted to the experimental data obtained in the adsorption of erythromycin at different concentrations, with a fixed sorbent concentration, at 30 °C (Figure 4). When the XAD-16N™, XAD-18™ and XAD-1600N™ sorbents were used, the observed profiles suggest a good fit for both Freundlich (eq. 3) and Langmuir (eq. 4) models to the experimental adsorption data (Figure 4), the estimated parameters by non-linear regression from the respective equations are presented in Table 3.

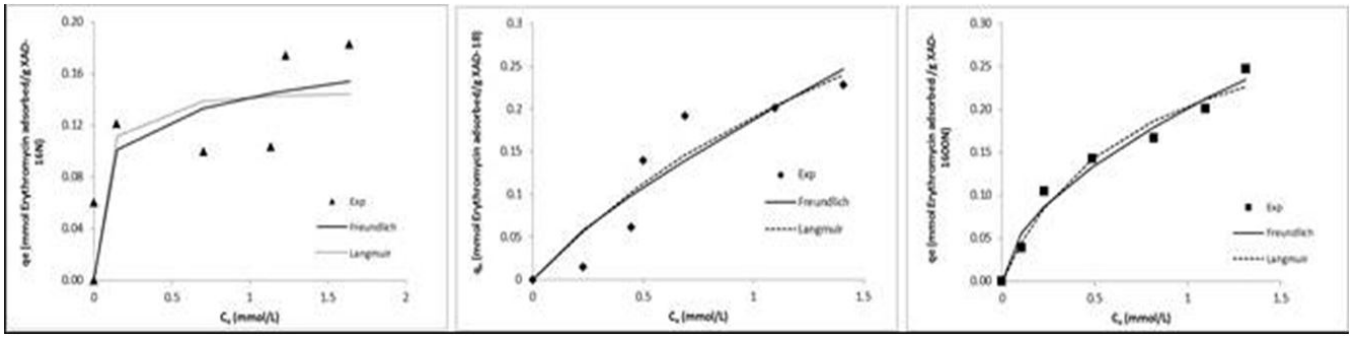


**Figure 4:** Sorption isotherms (Freundlich and Langmuir models), at 30°C, of erythromycin adsorbed to the different sorbent: XAD-16NTM, XAD-18TM and XAD-1600NTM

| Sorbent | Freundlich Model | | Langmuir Model | | |
|---|---|---|---|---|---|
| | $k_f$ | n | $q_{max}$ (mmol/g) | $k_L$ (mmol/L) | $R_L$ |
| XAD-16N™ | 0.14 | 0.17 | 0.15 | 0.05 | 0.04 |
| XAD-18™ | 0.19 | 0.80 | 0.64 | 2.32 | 0.77 |
| XAD-1600N™ | 0.20 | 0.57 | 0.35 | 0.70 | 0.46 |

**Table 3:** Estimated parameters of the isotherms models of Freundlich and Langmuir

The parameter, *n*, in the Freundlich model is less than 1 for all three polymers tested, which indicated that the erythromycin adsorption process is favorable. A greater, $k_f$ value, indicated a higher overall adsorption capacity. No significant differences were observed between the three microstructured materials.

For XAD-16N™ strong erythromycin adsorption at low sorbent concentrations, was observed (Figure 4).

With XAD-18™, the initial slope was convex to the axis of erythromycin concentration at equilibrium ($C_e$), followed by an inflection point which leads to an isothermal S-shaped sigmoid. This profile corresponds to a type IV isotherm according to the BET classification, also called stepwise isotherm, where each step corresponds to the formation of a well-defined layer of solute adsorbed on meso- or macroporous solid [28].

The $R_L$ values (Eq. 5) embedded in Table 3, are between 0 and 1 to the three polymers, reinforcing a favorable adsorption process.

Among the microstructured materials tested, XAD-16N™ showed better performance on the adsorption of erythromycin, since a lower n value, the higher energy of adsorption (related to $k_f$ values), a lower $k_L$ (from the Langmuir model) and $R_L$ (Table 4). [37] studied the adsorption of aqueous erythromycin (EM) to macroporous resin Sepabead SP825 in a series of batch experiments and they observed that the equilibrium data were well described by a Langmuir isotherm. [38] studied erythromycin separation from fermentation broth by resin adsorption–aqueous crystallization.

| Sorbent | $\Delta H_{ads}$ (kJ/mol) | $\Delta G_{ads}$ (kJ/mol) | $\Delta S_{ads}$ (J/mol.K) |
|---|---|---|---|
| XAD-16N™ | -3.68 | -52.47 | 16.10 |
| XAD-18™ | -2.56 | -1.10 | -4.80 |
| XAD-1600N™ | -2.72 | -3.60 | 2.90 |

**Table 4:** Thermodynamics parameters, sorption enthalpy (ΔHads), sorption free energy (ΔGads), and sorption entropy (ΔSads) on erythromycin sorption on different sorbents

## Thermodynamics Studies

### Estimation of Adsorption Thermodynamic Parameters

The effect of temperature on the adsorption of erythromycin onto neutral polymers XAD-16N™, XAD-18™, and XAD-1600N™ is illustrated in Figure 5. An optimum adsorption temperature at 50 °C for XAD-16N™ and XAD-1600N™ polymers was observed, while adsorption on XAD-18™ was favored at a temperature above 60 °C.

The enthalpy of adsorption was calculated, according to eq. 6 (Table 4). The values lower than 40 kJ/mol indicates that physical adsorption occurred [39].

For the systems where the Langmuir isotherm could be fitted to the experimental data (Table 4), free energy of adsorption was estimated by eq. 7. The negative values of $\Delta G_{ads}$ (Table 4) indicate the spontaneous nature of erythromycin adsorption to XAD-16N™, XAD-18™ and XAD-1600N™ and the positive values of entropy (eq. 8) shows the increased randomness after the adsorption of erythromycin on XAD-16N™ and XAD-1600N™ are rather low indicating that physisorption processes occurred [39].

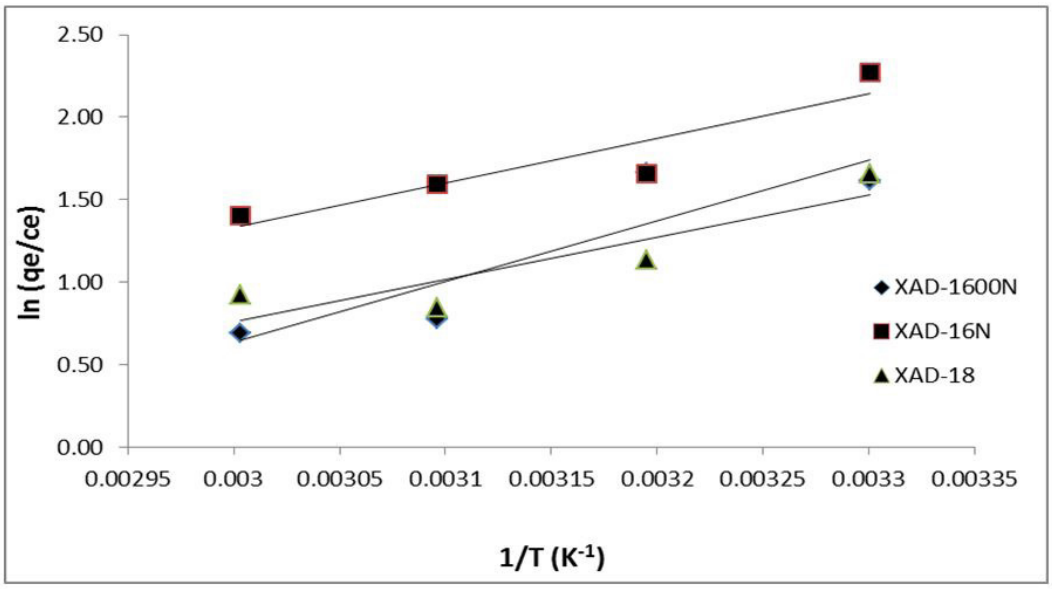


**Figure 5:** Representation of Van't Hoff equation on erythromycin sorption to XAD-16N™, XAD-18™ and XAD-1600N™

## Optimization of Erythromycin Adsorption onto Neutral Sorbents Using A Quality by Design Approach (Qdd)

Optimization of erythromycin recovery from production media onto the neutral polymeric sorbents, XAD-16N™, XAD-18™ and XAD-1600N™ was carried out using a quality by design approach, according to a CCRD as a function of time, temperature, and erythromycin and neutral polymeric sorbents concentrations (Table 1). The main and interactive effects of the four individual variables on the response (concentration of residual erythromycin in solution) were analysed [40]. The effect of autonomous variables on residual erythromycin in solution was assessed using a polynomial equation and 3D response surface plot constructed by the software as shown in Figures 6,7 and 8. The significant effects of time, temperature, [erythromycin] and [neutral polymeric sorbent] and the respective interaction on the erythromycin adsorption, were evaluated based on the residual erythromycin in solution, as shown in Table 5.

| Variables | Residual [Erythromycin] (mM) | | | | | |
|---|---|---|---|---|---|---|
| | XAD-16N | | XAD-18 | | XAD-1600N | |
| | | *(p)* | | *(p)* | | *(p)* |
| Time (Linear term) | -0.4002 | (0.0408*) | -0.3625 | (0.1627) | -0.7010 | (0.0126) |
| Time (Quadratic term) | 0.6777 | (0.0045**) | 0.5051 | (0.0343*) | 0.4904 | (0.0123*) |
| Temperature (Linear term) | -0.4208 | (0.0333*) | -0.4592 | (0.1018) | -0.3867 | (0.0594) |
| Temperature (Quadratic term) | 0.5628 | (0.0124*) | 0.3902 | (0.0646) | 0.2461 | (0.0726) |
| [Sorbent] (Linear term) | -0.6053 | (0.0057**) | -0.6321 | (0.0155*) | -0.8264 | (0.0022**) |
| [Sorbent] (Quadratic term) | 0.5053 | (0.0208*) | 0.1458 | (0.3636) | 0.3035 | (0.0439*) |
| [Erythromycin] (Linear term) | 1.6046 | (0.0000***) | 1.1360 | (0.0103**) | 1.0635 | (0.0039*) |
| [Erythromycin] (Quadratic term) | 0.4334 | (0.0400*) | 0.1027 | (0.5063) | -0.0126 | (0.8978) |
| Time x Temperature | -0.3461 | (0.5564) | -1.1099 | (0.0229*) | -0.7556 | (0.0214*) |
| Time x [Sorbent] | 0.0610 | (0.8681) | 0.1524 | (0.4249) | -0.0915 | (0.4657) |
| Time x [Erythromycin] | -0.2307 | (0.6897) | -0.2865 | (0.3464) | -0.3969 | (0.1024) |
| Temperature x [Sorbent] | -0.0813 | (0.8252) | 0.3963 | (0.0963) | 0.3354 | (0.0552) |
| Temperature x [Erythromycin] | 0.0643 | (0.9102) | 0.0541 | (0.8468) | -0.2436 | (0.2484) |
| [Sorbent] x [Erythromycin] | -0.0813 | (0.8252) | -0.1118 | (0.5477) | -0.2947 | (0.0747) |

T* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

**Table 5:** Effects and respective significance levels (*p*) of time, temperature, [neutral polymeric sorbent], and [Erythromycin] on erythromycin sorption, evaluated as residual erythromycin in solution (not adsorbed) (mM)

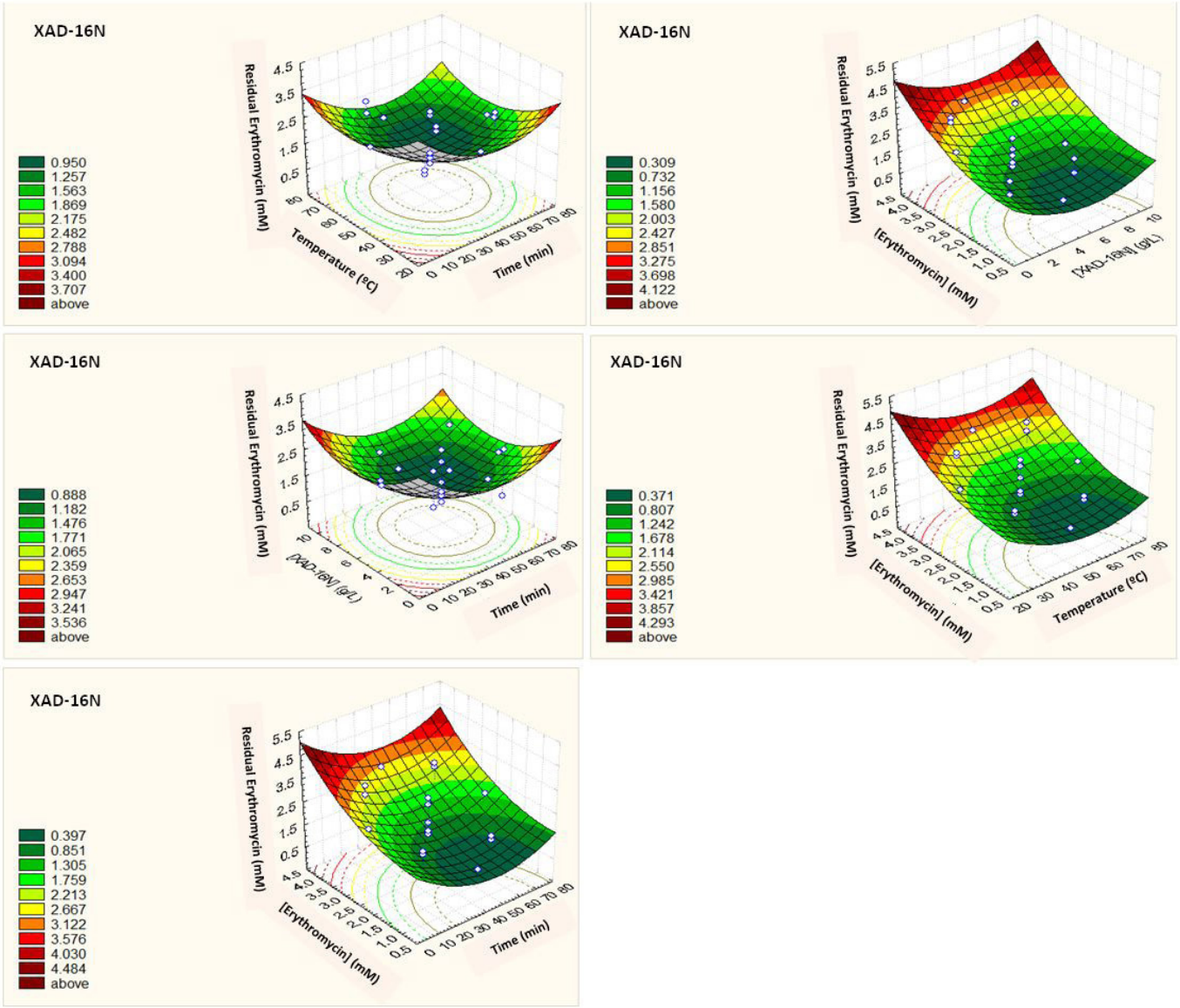


**Figure 6:** 3D response surface plot and fitted surface adjusted to the experimental data in sorption process to XAD-16N™, corresponding to the concentration of residual erythromycin in solution (non-adsorbed) (mM) as a function of temperature (ºC), time (min), concentration of XAD-16N™ (g/L) and concentration of erythromycin (mM)

When XAD-18 and XAD-1600N are used, multiple regression coefficients, obtained by employing a least squares technique to predict a quadratic polynomial model for residual erythromycin in solution, with the t-test indicated that quadratic terms of time and temperature, as well as all neutral polymeric sorbents (linear terms), were highly significant, $p < 0.01$, $p < 0.001$ and $p < 0.001$, respectively (Table 5).

The interactions between the variables time and temperature were significant ($p < 0.05$) when XAD-18 and XAD-1600N were used. A negative interaction, time–temperature indicated that higher adsorption of erythromycin can be obtained at higher time at moderate temperatures [41].

Furthermore, the interactive effect of variables was also determined with the help of a 3D response surface plot.

As depicted in Figure 6, the residual erythromycin concentration decreased which means higher adsorption to XAD-16N™, at moderate temperatures and reduced contact time. In the adsorption process of erythromycin on the XAD-16NTM, contact time, linear and quadratic terms, have a significant effect, as well as the linear and quadratic terms of temperature, [XAD-16N™] and [erythromycin] (Table 5).

In Figure 7 is presented, the results of the adsorption of erythromycin to XAD-18. High levels of erythromycin are adsorbed at lower times [42].

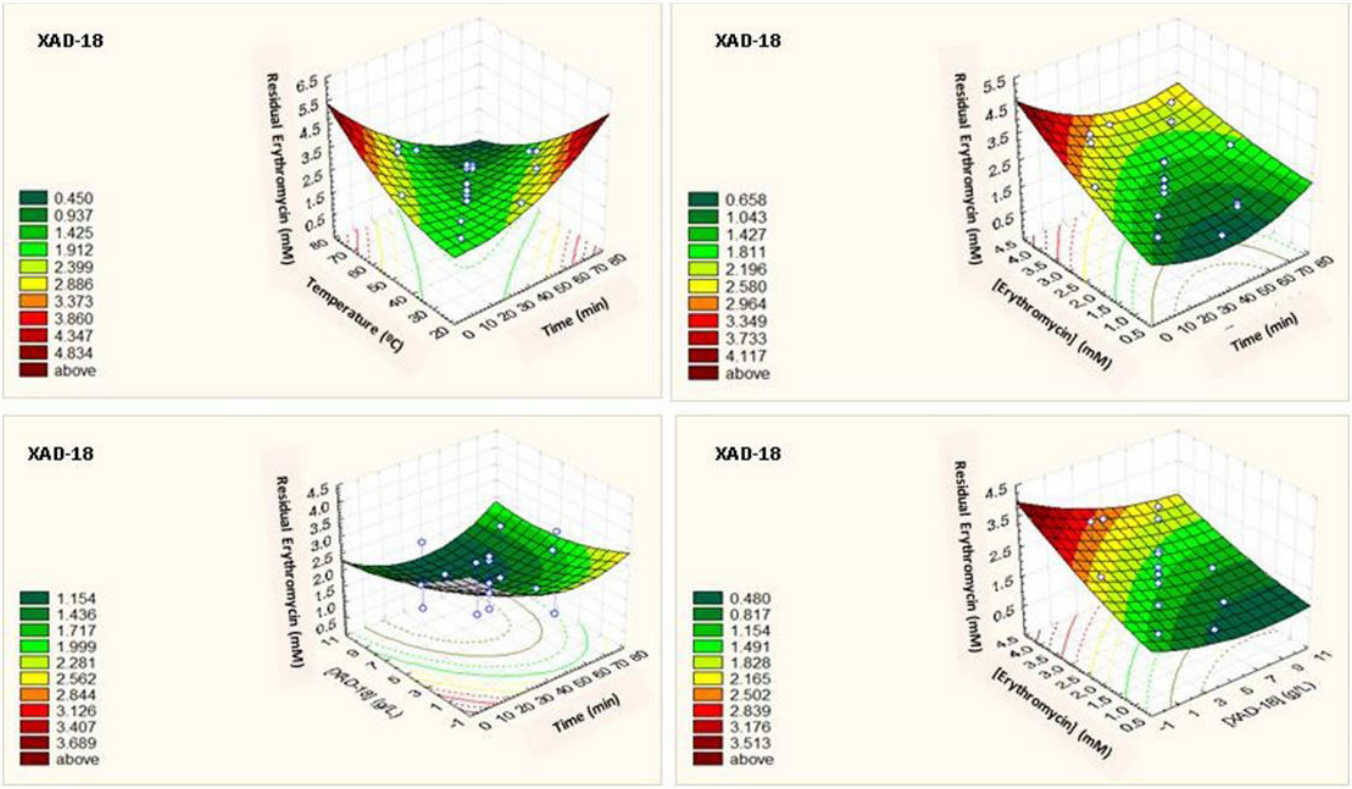


**Figure 7:** 3D response surface plot and fitted surface adjusted to the experimental data in sorption process to XAD-18™, corresponding to the concentration of residual erythromycin in solution (non-adsorbed) (mM) as a function of temperature (ºC), time (min), concentration of XAD-18™ (g/L) and concentration of erythromycin (mM)

In the adsorption of erythromycin on XAD-1600N™, the contact time, a linear term, has a significant effect, as well as the linear terms of [XAD-1600™] and [erythromycin], the quadratic term of [XAD-1600™] and the interaction between time x temperature (Table 6).

A greater amount of erythromycin was adsorbed with sorbent concentrations close to 5 g/L (Figure 8).

The experimental results obtained were adjusted to 2nd order polynomial equations (Table 6) describing the response surfaces of the four variables considered. The proportion of the total variation assigned to each setting can be measured by the value of the squared correlation coefficient ($R^2$). $R^2$ values greater than 0.75 are indicative of a good adaptation to the model. In this work, all the sorbents tested in erythromycin recovery obtained $R^2$ greater than 0.94, in particular for XAD-18™ and XAD-1600N™, $R^2$ were 0.99, and respectively, 0.94 and 0.97 for $R^2_{adj}$ (Table 6). These values indicated a good fit of the models to the experimental results [43].

| Sorbent | Model Equations | $R^2$ | $R^2_{adj}$ |
|---|---|---|---|
| XAD-16N™ | $[Erythromycin]_{Residual}$ = 7.0106 – 0.0778Time – 0.00085 $Time^2$ – 0.1391Temperature + 0.0013$Temperature^2$ – 0.4849[XAD-16N] + 0.0404$[XAD-16N]^2$ – 0.28124 [Erythromycin] + 0.2167$[Erythromycin]^2$ | 0.939 | 0.885 |
| XAD-18™ | $[Erythromycin]_{Residual}$ = 0.00063$Time^2$ – 0.00185 Time x Temperature + 0.00447Temperature x [XAD-18] | 0.989 | 0.941 |
| XAD-1600N™ | $[Erythromycin]_{Residual}$ = 0.0006$Time^2$ – 0.4235[XAD-1600N] + 0.0243 $[XAD-1600N]^2$ – 1.6316[Erythromycin] - 0.0013Time x Temperature + 0.00447Temperature x [XAD-1600N] | 0.995 | 0.969 |

**Table 6:** Second-order model equations for the response surfaces fitted to the experimental data points, as a function of Time, Temperature, [Sorbent] and [Erythromycin], respectively, and coefficient of determination, $R^2$, and $R^2_{adj}$. The dependent variable was the residual [Erythromycin] (not adsorbed)

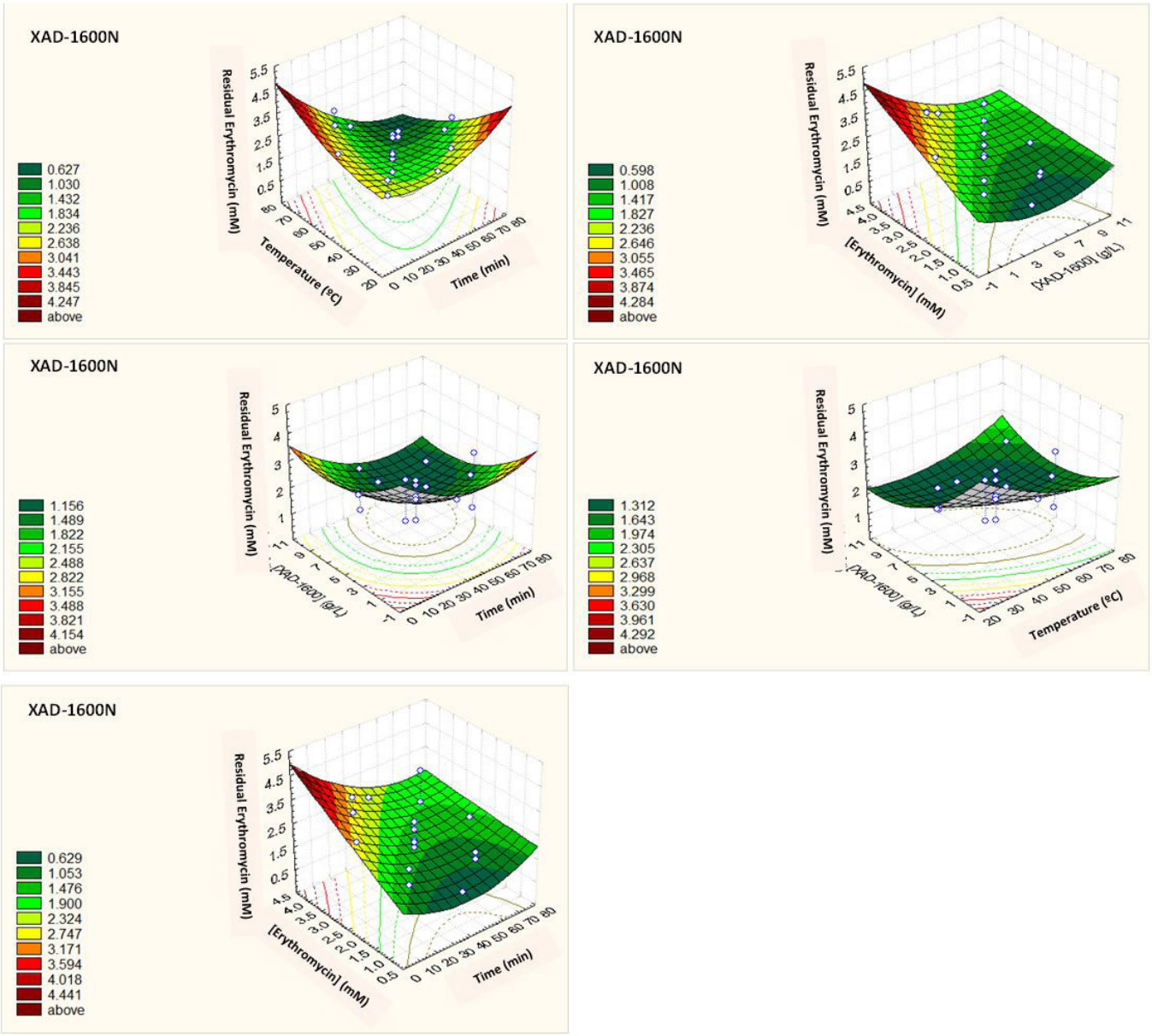


**Figure 8:** 3D response surface plot and fitted surface adjusted to the experimental data in sorption process to XAD-1600N™, corresponding to the concentration of residual erythromycin in solution (non-adsorbed) (mM) as a function of temperature (ºC), time (min), concentration of XAD-1600N™ (g/L) and concentration of erythromycin (mM)

## Conclusions

Erythromycin produced by *Saccharopolyspora erythraea* was successfully adsorbed to neutral microstructured materials.

A microscale approach was successfully used in this work. All the neutral microstructured materials showed high erythromycin adsorption efficiency. XAD-16N™ and XAD-18™ showed the best results (> 80% adsorption). The estimation of the n and $R_L$ parameters of the Freundlich and Langmuir isothermal models, as well as the values of the thermodynamic parameters ($\Delta H < 40$ kJ/mol, negative values of $\Delta G$, and the positive values of $\Delta S$ for XAD-16N™ and XAD-1600N™) showed that the adsorption of erythromycin to the three sorbents is favorable and indicates that a physio adsorption process occurred.

High adsorption rates were obtained with low sorbent concentrations (close to 5 g/L) and a reduced increase of adsorption occurred with the increase in temperature. For each erythromycin/sorbent system tested, the adsorption equilibrium was reached before 60 min contact time. Adsorption of erythromycin from production media was successfully optimized using a quality by design approach.

This adsorption process is gaining considerable prominence in the pharmaceutical biotechnology industry, especially due to the new therapeutic indications of erythromycin. Additionally, this adsorption process could be applied to remove low quantities of erythromycin from wastewater.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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