



Modelling of the rinsing of a Fixed Bed Reactor for Solid Phase Peptide Synthesis using COMSOL Multiphysics®

Raphaël Bayle^{1,*}, Jean-David Wheeler¹, Renan Ravetti², Patrick Namy¹, Olivier Ludemann-Hombourger²

¹SIMTEC, 5 rue Felix Poulat, 38000 Grenoble, France

²POLYPEPTIDE GROUP, 7 rue de Boulogne, 67100 Strasbourg, France

*Corresponding author. Email address: raphael.bayle@simtecsolution.fr

Abstract

Solid Phase Peptide Synthesis (SPPS) requires finely designed reactor. While the process itself is tuned during a lab-scale procedure, the scale-up to production size reactor opens new challenges. The efficiency of the reaction and the quality of the final substance can be made like the lab-scale results, but it requires to master the critical scale up parameters. In this study, the process design is secured by using a numerical model. The fluid flow in the whole reactor and more specifically in the fixed resin bed during the draining steps is modelled in a realistic way through Brinkman equation and the appropriate boundary conditions. Using this computed fluid flow, the rinsing of the reactor can be studied by modelling the reagents to eliminate with particles whose trajectories are driven by the previously computed fluid flow.

Keywords: Fixed bed modelling, porous media, CFD, Peptides

1. Introduction

The peptide market has been strongly growing over the last decades. Peptide demands can vary a lot ranging from few milligrams demand for personalized therapy, to several hundreds of kilograms with the emergence of oral formulation.

Since the first peptide syntheses by Curtius (1881) and Fischer (1902), tremendous progress has been made in the chemical process. At first, the understanding of protein structure (by Hofmeister (1902) and Fischer (1906)) provided tools to think the process and its improvement. A milestone in the field was the total synthesis of oxytocin in 1953 (Du Vigneaud et al., 1953), which opened the path for the development of new industrial strategies for peptide synthesis and the development of new applications of peptides as active pharmaceutical ingredients. Originally based on solution-phase production

techniques (Bray et al., 1990), the production was tedious to produce long peptide sequences, until the emergence of Solid Phase Peptide Synthesis (SPPS) (Merrifield, 1963), which became quickly the reference technology used to produce peptides. The latter allows for simplifying the production steps but is however restricted to intermediate production size.

The increasing demand for large scale manufacturing requires a scale up of the SPPS reactors, with a technical challenge to maintain the efficiency of the process. The design of the reactor should be mastered to keep the best performance, primarily driven by the process productivity and by the solvent consumption. SPPS is widely used for the manufacturing of synthetic peptides, but requires large quantities of hazardous solvents, such as N,N-dimethylformamide (DMF).

A new washing practice has been recently proposed, to drastically reduce the solvent consumption: the solid phase reaction is performed in a stirred bed reactor



equipped with a bottom frit, and the removal of the reagents is performed by a percolation by the rinsing solvent through a fixed bed of the resin after stopping the stirrer and sedimentation of the resin (Ravetti Duran & Ludemann-Hombourger, 2022).

Even if the enlargement of reactor seems promising, it also brings new challenges: as the dimension are increased, the rinsing step in the process has to be carefully studied in order to guarantee that the reagents are efficiently removed.

Numerical modelling makes it possible to simulate physical system and assess their performance to secure the design. Numerical modelling is used here to assess the rinsing efficiency during the percolation process and to understand the key parameters influencing the removal of the reagents from the resin. The aim of this paper is to present a methodology to study the rinsing of a fixed bed reactor filled with a resin behaving as a porous medium and to develop an efficient and robust technical solution.

2. State of the art

Fluid flow in porous media has been thoroughly studied (Nield & Bejan, 2017). Among the various existing models, the Brinkman equations extends the Darcy's law to fast moving fluid and makes it possible to consider viscous dissipation (Le Bars & Worster, 2006)

To track the convection of chemical species, one can chose conventional convection dominated equation schemes but they are prone to generate numerical issues with finite element methods (Habchi et al., 2008) and require stabilization techniques such as artificial diffusion (Zienkiewicz et al., n.d.) or Galerkin Least Square formulation (Hughes et al., 1989). Therefore the authors favoured particle tracing as it is a convenient way to track objects whose trajectories are determined by various phenomena such as fluid flow (Strubel, 2016).

The application of computational fluid dynamics in porous media coupled with particle tracing to the study of the rinsing of a solid phase peptide reactor is, to the best of our knowledge, a new step towards designing optimized reactor.

3. Materials and Methods

In this section, the developed methodology to assess the rinsing efficiency of the reactor and its implementation are described.

3.1. Developed model

In this first subsection, the model that has been developed is presented. The geometry of the modelled system, the implemented physics and the parameters are described; a solution to some numerical challenges underlying the model is presented.

3.1.1. Geometry

The modelled system is composed of a reactor filled with a resin. The reactor is equipped with a stirrer, which is static during the rising step. The bottom of the reactor, in grey in Figure 1, is composed of a frit to retain the resin and of a distribution chamber composed by an alveolate layer. Note that in the following, it is assumed that, above the explicitly represented resin bed, a layer of liquid of constant height is present.

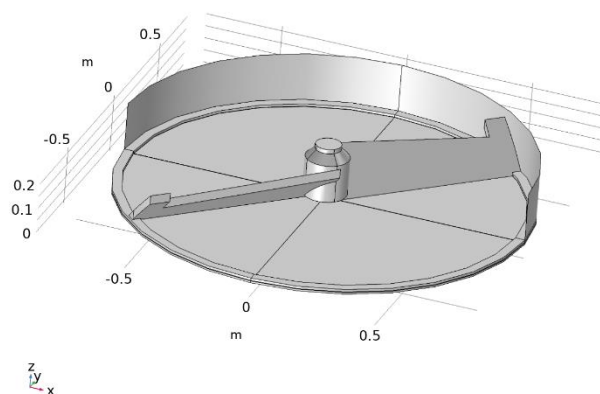


Figure 1 : Geometry of the reactor. For better readability, the porous media filling the reactor as well as the tank wall from the camera side are not represented here.

3.1.2. Implemented physics and boundary conditions

All the domain except the reactor wall and the agitator are considered as porous media.

The fluid flow in the porous media is represented using the stationary Brinkman equations under the Stokes flow hypothesis:

$$\begin{aligned} \nabla \cdot \left(-pI + \frac{\mu}{\epsilon_p} (\nabla u + (\nabla u)^T) - \frac{2}{3} \frac{\mu}{\epsilon_p} (\nabla \cdot u) I \right) - \frac{\mu}{\kappa} u &= 0 \\ \nabla \cdot (u) &= 0 \end{aligned} \quad (1)$$

where p is the pressure field, u the velocity field, μ the fluid viscosity, ϵ_p the medium porosity, ρ the fluid density and κ the medium permeability.

Boundary conditions for those equations should be specified. The boundary conditions are a fixed pressure at the top boundary of the system, see Figure 2, and a fixed normal velocity at the exit of the system, see Figure 3; the normal velocity u_0 at the exit of radius r_{exit} of the system is fixed using the flow rate D ,

$$u_0 = D / (2\pi r_{exit}^2). \quad (2)$$

Other boundary conditions are slip conditions,

$$u \cdot n = 0, \quad (3)$$

meaning that no fluid can penetrate those walls but

that the tangential velocity is not necessarily zero.

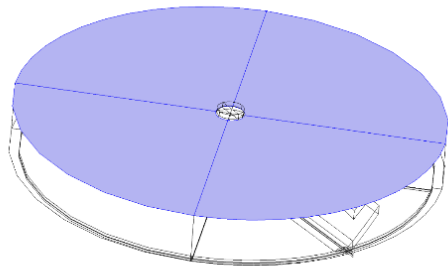


Figure 2 : top boundary condition where a constant pressure is prescribed.

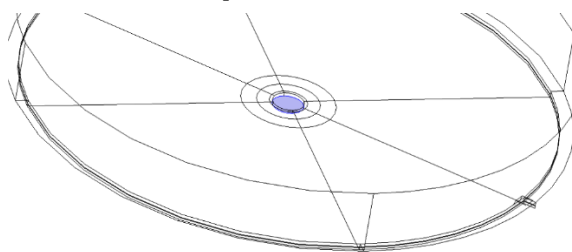


Figure 3 : bottom boundary, in blue, on which the exit flow rate is prescribed. The agitator has been removed of this figure for visualisation purposes.

The calculation of the reagent exiting flux is carried out by tracking particles transported by the fluid whose velocity is determined by the stationary Brinkman equations previously presented. As stated in section 3.1.1, a layer of fluid is implicitly considered on the top of the resin bed. Initially the concentration of the reagent in this layer is the same as in the resin; this concentration will decrease as pure solvent, without reagent, will be added by percolation. Thus, two contributions must be considered: a first one due to the reagent coming from the liquid layer above the porous medium and a second one due to the reagent initially present in the porous medium.

Those two contributions are modelled using particle trajectories obtained from two initial distributions. The first initial distribution is a surface distribution and the second one is a volume distribution, see respectively Figure 4 and Figure 5.

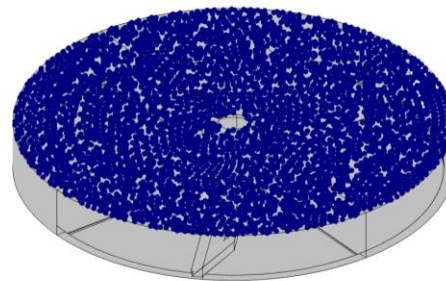


Figure 4 : Initial surface distribution from which F_{surf} is calculated.

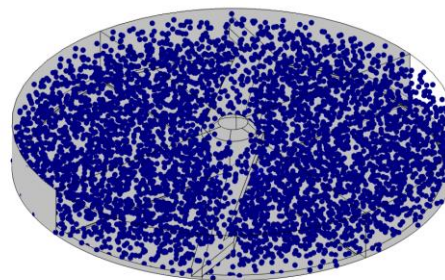


Figure 5 : Initial volume distribution from which F_{vol} is calculated.

The final evacuated reagent flux can be obtained with the following formula:

$$J_{out}(t_0) = n_0 * F_{vol}(t_0) + \int_{t < t_0} J_{in}(t) F_{surf}(t_0 - t) dt \quad (4)$$

here $J_{out}(t_0)$ (in mol/s) is the reagent flux toward the outside at t_0 , n_0 (in mol) is total reagent quantity initially present inside the porous medium (explicitly represented part), $F_{vol}(t_0)$ (in 1/s) is the fraction of particles initially in the porous medium going through the exit after a time t_0 , J_{in} (in mol/s) is the total reagent flux at the top surface, due to the reagent present in the liquid layer above the porous medium, and $F_{surf}(t_0)$ (in 1/s) is the fraction of particles initially at the surface going through the exit after a time t_0 .

Note that F_{surf} and F_{vol} are normalized so that their integral versus time varying from 0 to infinity is 1.

$J_{int}(t)$ is calculated from the concentration inside the fluid layer $c(t)$ and the flow rate D ,

$$J_{int}(t) = D * c(t). \quad (5)$$

Denoting D the mass flow rate, V the volume of the liquid layer above the resin bed, during a time dt a volume $D * dt$ of fluid at reagent concentration $c(t)$ in the tank is replaced by pure solvent. Assuming at time $t + dt$ the concentration is homogeneous, one can write:

$$c(t + dt)V = c(t)(V - D * dt) \quad (6)$$

which can be written

$$\frac{c(t + dt) - c(t)}{dt} = -\frac{D}{V}c(t). \quad (7)$$

At the limit $dt \rightarrow 0$, the following differential equation for concentration evolution is deduced:

$$\frac{dc}{dt}(t) + \frac{D}{V}c(t) = 0 \quad (8)$$

Denoting c_0 the initial concentration, one gets,

$$c(t) = c_0 e^{-\frac{D}{V}t}. \quad (9)$$

3.1.3. Parameters

The parameters of the simulations associated with the porous media properties are presented in Table 1; those associated with fluid and chemical properties are presented in Table 2.

Table 1. Porous media parameters

Parameters	Value	Unit
Resin porosity	0.37	\emptyset
Resin permeability	5.51E-12	m^2
Filter porosity	0.62	\emptyset
Filter horizontal permeability	5.7187E-9	m^2
Filter vertical permeability	2.9032E-12	m^2
Alveolate layer porosity	0.8	\emptyset
Alveolate layer permeability	$10^{-8} m^2$	m^2

Table 2. Fluid and chemical parameters.

Parameters	Value	Unit
Fluid viscosity	0.867	$mPa.s$
Fluid density	944	$kg.m^{-3}$
Flow rate	4	m^3/h
Initial concentration	2022.2	mol/m^3
Liquid layer volume	0.080425	m^3

3.1.4. Numerical challenges

As previously stated, particles are driven by the velocity field. As numerical error slightly deviates them, some particles may collide with the walls. To avoid such

spurious collisions to happen, two modifications have been implemented. The first modification is related to the mesh: introducing boundary layers reduced the number of collisions. A 2D cut of the mesh including boundary layers is given in Figure 6. The second modification is that the velocity field from the Brinkman equations is modified so that if a particle is too close to the wall and moving towards it, the normal component of the velocity in the wall referential is deleted. Denoting u the velocity field obtained by resolution of Brinkman equations, the particles are now driven by a field \tilde{u} defined by

$$\tilde{u}(x, t) = u(x, t) - (u(x, t) \cdot n) n * (u(x, t) \cdot n < 0) * (d(x) < \epsilon) \quad (10)$$

where $d(x)$ is the distance between a given point x and the closest wall, ϵ is a numerical parameter indicating the minimal distance so that the normal component of the velocity is deleted and $n = \nabla d$ is the normal to the wall, computed using the gradient of the wall distance function.

Physically, this second modification is justified because even if theoretically the distance between the particles and the walls could be zero, because of diffusion, a particle cannot be confined just at the wall interface. The characteristic diffusion length L can be estimated using $L = \sqrt{D_{chemical}T}$ where $D_{chemical}$ is the chemical diffusion and T is a characteristic time of the system. Choosing $D = 10^{-9} m^2/s$ and a time $T = 10 s$, one obtains a characteristic diffusion length of $10^{-4} m$. Thus, if the velocity field is modified with $\epsilon = 10^{-4} m$, diffusion ensures that no error is made.

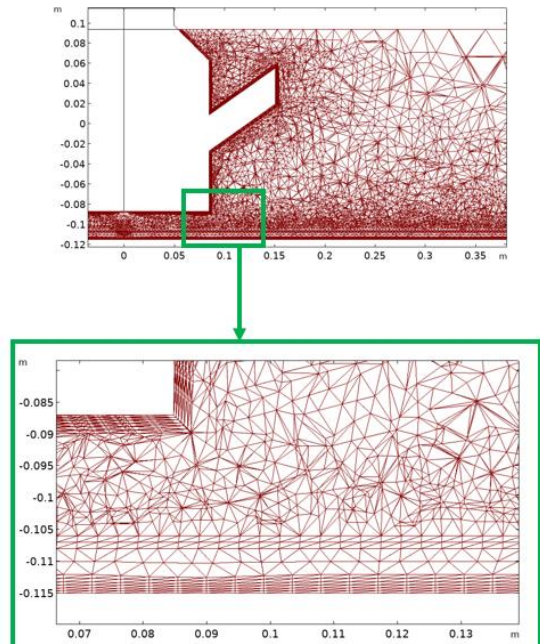


Figure 6 : vertical 2D cut of the mesh where boundary layers are added on the walls.

3.2. Numerical method

The aim of the present work is the rinsing of a resin bed reactor by percolation, using sets of partial differential equations (PDE) to compute the fluid flow and then track particles accounting for the reagent that are driven by the fluid flow.

Numerical resolution of PDE can be done with different numerical methods. Among the most widely used is the finite element method (FEM), which provides accurate approximations of model solutions and is particularly efficient when complex geometries are involved.

The software COMSOL Multiphysics®, versions 5.6, is used for numerical computations in the present study. This software is based on the finite element method which makes it possible to implement efficiently various physics such as heat, mass and momentum transport equations. One of the major strengths of COMSOL Multiphysics® is to provide a unified framework where those various physics can be coupled in order to solve multi-physics problems that appear in complex systems, especially in industrial ones. In particular COMSOL Multiphysics® integrates a particle tracing module that can be coupled with fluid dynamics computation. Beyond the elaboration of models, COMSOL Multiphysics® makes it possible to solve them numerically and integrates postprocessing tools to visualize simulation results.

4. Results and Discussion

In this section, the results of the model presented in section 3, are presented and discussed.

4.1. Results

Following the previously described modelling, the first step to obtain the evolution of the total quantity of reagent inside the system is to solve the Brinkman equations to obtain the velocity field inside the porous medium. The result of such a computation is shown in Figure 7.

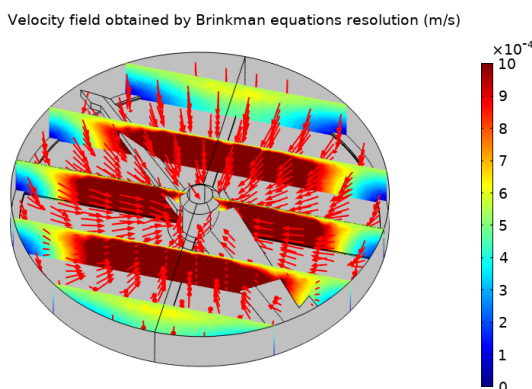


Figure 7 : Velocity field obtained by solving the Brinkman equations. The color scale unit is m/s. The arrow represents the normalized velocity direction, in the slices, the velocity norm is displayed using a color range from 0 to 1 mm/s.

From the calculated velocity field inside the porous medium, the number of particles exiting the reactor per second can be calculated for both an initial surface distribution and an initial volume distribution, as previously illustrated in Figure 4 and Figure 5. This number of exiting particles per second is shown in Figure 8: as expected for surface initial particle distribution case, the particles should first elute through the resin bed before exiting the reactor and a peak is measured at around 400 s; while for the volume initial distribution case, a steady flux can be observed with a decrease at around 400 s.

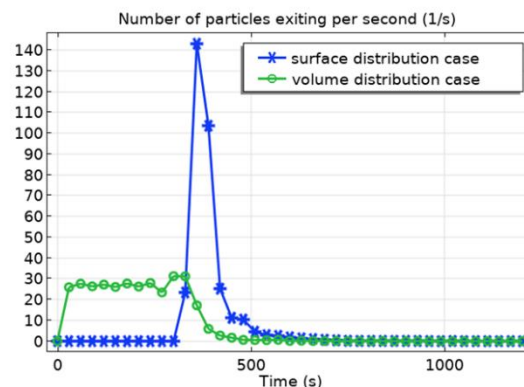


Figure 8 : Number of particles exiting the reactor per second for both initial surface distribution (blue curve) and initial volume distribution from which function F_{surf} and F_{vol} are calculated.

From the exiting flux of particles for both surface and volume initial distribution, the exiting reagent molar flux can be computed using introduced formula and notation introduced in section 3.1.2,

$$J_{out}(t_0) = n_0 * F_{vol}(t_0) + \int_{t < t_0} J_{in}(t) F_{surf}(t_0 - t) dt. \quad (11)$$

The total reagent flux and both the volume contribution and the contribution from the liquid layer above the resin (upper tank) are presented in Figure 9. The contribution from the liquid layer (upper tank) is associated with the term $\int_{t < t_0} J_{in}(t) F_{surf}(t_0 - t) dt$ while the volume contribution is associated with the term $n_0 * F_{vol}(t_0)$.

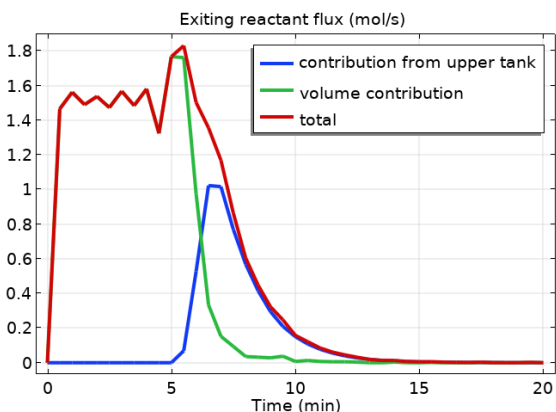


Figure 9 : Exiting reagent flux. The total contribution is the sum of the contribution due to the reagent initially present in the liquid layer above the resin (upper tank) and the contribution for the reagent initially inside the resin (volume contribution).

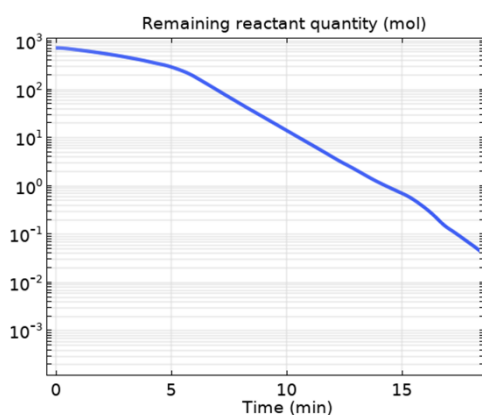


Figure 10 : Remaining reagent quantity as a function of time.

The remaining quantity of reagent as a function of time $n(t)$ can be easily calculated from the obtained flux $n(t) = n_0 - \int_0^t J_{out}(t') dt'$. The result is shown in Figure 10. The rinsing time can be defined according to the rinsing dilution criteria. For instance, with a 1/2000 dilution target, the solvent quantity would be equal to 2.65 resin bed volumes, which is similar to the achieved performance obtained on a pilot unit at smaller scale.

4.2. Discussion

The results of the simulation obtained with the implemented methodology proves that the current work enables to predict rinsing efficiency of a reactor with reasonable accordance to experimental data provided by pilot unit.

As described in section 3.1.4, numeric artefacts arise in trajectories calculation when the velocity field from the Brinkman equations is directly used: due to accumulated numerical errors, particles may collide with the boundary of the domains where no velocity are defined. It is worth noting that those artefacts are due to computation time constraints and are not inherent to the method. If the time step is reduced enough and

the particles initial state is fixed, it is theoretically possible to avoid the wall collision without modifying the velocity field. In the presented work, the choice to modify the velocity field is made to reduce the computational cost.

Although the remaining reagent quantity represented in Figure 10 is a smooth function of time, its derivative presented in Figure 9 exhibits fluctuations. This limitation is due to the finite number of particles. As with the decrease of the timestep to avoid collision, getting improvement on this point is computationally costly. Let us note that, since the particle trajectories are independent from one another and only depending on the velocity field calculated from Brinkman equations, the total trajectories computation time is a linear function of the number of particles.

To avoid inaccuracy due to a finite number of particles, a further work could be to compare the developed model with a model where the concentration of reagents is directly obtained from concentration field calculated from a diffusion-convection equation.

Two advantages would be associated with a model with such diffusion-convection equation: the first advantage would be that the interest quantity would be directly accessible by integration of the concentration field; the second advantage would be that location of areas where the reagents are the highest could be easily spotted. Nevertheless, despite those advantages, the convergence of such a model could be difficult, as the diffusivity in liquid is low. Such difficulty in reaching convergence could lead to the necessity of arbitrary raising the diffusion coefficient, which would spuriously decrease the rinsing time.

5. Conclusions

Reducing the solvent consumption in solid phase peptide synthesis is a challenge that has been addressed by the new washing practice of washing by percolation (Ravetti Duran & Ludemann-Homburger, 2022). The present work proposes a first model of this innovative rinsing process. It focuses on developing a methodology to assess the rinsing capability of a reactor filled with a porous medium and where the solvent is sucked up from the bottom: a mathematical model to reconstruct the total quantities of reagent in the system as a function of time has been developed.

Coupling Brinkman equations and a Lagrangian approach where virtual reagent particles are tracked has been demonstrated to enable prediction on the rinsing capacity of the process. This model estimates the quantity of reagent in the reactor as a function of time and thus, given a dilution target, it enables to predict the required solvent quantity and the dilution time.

In the aftermath of this work, a comparison between the proposed approach, where reagent quantity is

estimated from particle tracing, and an approach using a convection–diffusion equation to track the reagent concentration could be carried out. More comparisons with large scale reactor experimental data have also to be done.

Nevertheless, the developed methodology is already useful to assess and compare the rinsing efficiency of various reactor design: industrialists are interested in designing reactor where reagents can be easily evacuated, using as little solvent as possible. From the presented methodology, various geometrical modifications can be performed to optimize the rinsing process. For example, from the developed model, it is possible to study the impact of different collector geometries and rotor geometries, or the impact of a modification of the ratio between the radius and the height of the reactor.

References

- Bray, A. M., Joe Maeji, N., & Mario Geysen, H. (1990). The simultaneous multiple production of solution phase peptides; assessment of the geysen method of simultaneous peptide synthesis. *Tetrahedron Letters*, 31(40), 5811–5814. [https://doi.org/10.1016/S0040-4039\(00\)97966-8](https://doi.org/10.1016/S0040-4039(00)97966-8)
- Curtius, T. (1881). . *J. Prakt. Chem.*, 24, 239.
- Du Vigneaud, V., Ressler, C., Swan, J. M., Roberts, C. W., Katsoyannis, P. G., & Gordon, S. (1953). The Synthesis of an Octapeptide Amide With The Hormonal Activity of Oxytocin. *Journal of the American Chemical Society*, 75(19), 4879–4880. https://doi.org/10.1021/JA01115A553/ASSET/JA01115A553.FP.PNG_V03
- Fischer, E. (1902). . *Ber. Dtsch. Chem. Ges.*, 35, 1095.
- Fischer, E. (1906). . *Ber. Dtsch. Chem. Ges.*, 39, 530.
- Habchi, W., Eyheramendy, D., Vergne, P., & Morales-Espejel, G. E. (2008). A full-system approach to the elasto-hydrodynamic line/point contact problem. *Journal of Tribology*, 130(2), 21501–21510.
- Hofmeister, F. (1902). . *Physiol. Biol. Chem. Exp. Pharmacol.*, 1, 759.
- Hughes, T. J. R., Franca, L. P., & Hulbert, G. M. (1989). A new finite element formulation for computational fluid dynamics: VIII. The galerkin/least-squares method for advective–diffusive equations. *Computer Methods in Applied Mechanics and Engineering*, 73(2), 173–189. [https://doi.org/10.1016/0045-7825\(89\)90111-4](https://doi.org/10.1016/0045-7825(89)90111-4)
- Le Bars, M., & Worster, M. G. (2006). Interfacial conditions between a pure fluid and a porous medium: implications for binary alloy solidification. *Journal of Fluid Mechanics*, 550, 149–173. <https://doi.org/10.1017/S0022112005007998>
- Merrifield, R. B. (1963). Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide. *Journal of the American Chemical Society*, 85(14), 2149–2154. https://doi.org/10.1021/JA00897A025/ASSET/JA00897A025.FP.PNG_V03
- Nield, D. A., & Bejan, A. (2017). Convection in porous media. *Convection in Porous Media*, 629–982. <https://doi.org/10.1007/978-3-319-49562-0/COVER>
- Ravetti Duran, R., & Ludemann-Hombourger, O. (2022). SPPS: process improvements to reduce solvent consumption. *Spec Chem Mag*, 40–43.
- Strubel, V. (2016). Particle Entrapment in EHD Contacts – Aerospace Applications. *Doctoral Dissertation, INSA de Lyon*.
- The Finite Element Method: Solid mechanics* – O. C. Zienkiewicz, Robert Leroy Taylor, R. L. Taylor, Robert Lee Taylor – Google Livres. (n.d.).