Polymeric Carriers – The Influence of Body Fluid Compounds on a Drug Local Release

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Keywords: Polymeric Drug Carrier, Diffusion, Mass Transport Coefficient, Body Fluid Compounds, Local Therapy.

Abstract: The release of a model compound (cyanocobalamin) from the core-shell structure of drug carrier was considered. Mass transfer was described by the classical equation describing a diffusion mass flow. The presence of compounds found in body fluids on the diffusional mass stream was investigated. It has been shown that high molecular weight compounds unable to penetrate the carrier surface form on the carrier surface a layer that slightly slows the drug release. Slowing down also occurs in the case of counter-current transport of soluble organic components. However, the salts (particularly NaCl) present in body fluids, probably due to the emerging osmotic pressure, significantly accelerate the transport of the released drug. In order to prevent this phenomenon, salts at the concentrations equal to their concentrations in the fluid surrounding the carrier should be placed into the carrier.

1 INTRODUCTION

Drug delivery systems based on polymers have been studied over the last ten years in the aspect of achieving high therapeutic concentrations of anti-tumor drugs in local therapies (Davis et al., 2008; Weinberg et al., 2008; De Souza et al., 2010). The development of these technologies is fueled by increasing the bioavailability of the drug at the disease site, delivering it to cancer tissues, increasing its solubility and minimizing side effects. Existing systems can be divided into two groups depending on how they are delivered and how they work.

The first consists of systemic delivery and is based primarily on nanomaterials such as polymer nanoparticles, liposomes and dendrimers. Such carriers have the function of actively locating the target site by coupling them to various chemical species that have a strong affinity for markers of tumor expression or by releasing the mass as a result of responses to localized stimuli (pH, temperature, etc.) (Wolinsky et al., 2012; Klinkier, 2017; Li et all, 2017).

The second group includes the polymeric carriers located at the site of the cut tumor or adjacent to cancerous tissue. These solutions have been implemented in a variety of forms including films, gels, plates and particles (Langer, 1983). Polymers used in these systems are of natural origin (Al-Ghananeem et al., 2009; Gerber et al., 2011; Li et al., 2011; Barhoumi et al., 2015; Kulkarni et al., 2015), however also synthetic polymers are used (Wolinsky et al., 2012). They are usually not biodegradable but biocompatible.

The application of the second group of carriers is to release the active ingredient at a strictly defined daily dose, at a relative steady stream value. The previous paper (Trusek-Holownia and Jaworska, 2012) presented the model of a drug diffusive transport from the core-shell type carriers. The overall mass transport coefficient is applied in this model.

Considering the resistances of mass transport, it has been accepted and verified (Trusek-Holownia and Jaworska, 2014) that the dominant resistance is derived from the transport through the membrane. Therefore, the value of the coefficient strongly depends on a membrane thickness, its porosity, a pore size. It is also a function of the type of substance (mainly it's diffusion coefficient and molecule size) and the type of environment (the medium filled the pores of the membrane).

In presented work an additional parameter was considered, which may influence on the mass flow rate. The influence of compounds present in body fluids surrounding the carriers has been studied. As these carriers are administered at a site that has been

Trusek-Holownia, A. and Latka, A.
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DOI: 10.5220/0006657202410246
In *Proceedings of the 11th International Joint Conference on Biomedical Engineering Systems and Technologies (BIOSTEC 2018) - Volume 1: BIODEVICES*, pages 241-246
ISBN: 978-989-758-277-6

changed by disease (inflammation) or after removal of the tumor, the somatosensory efflux is a common occurrence. That why the compounds present in lymph were considered.

Lymph consists mainly of intrahepatic fluid, but in relation to it there is a much higher concentration of organic matter, fats and immune cells (leukocytes). The composition of a lymph depends on where it originated and how inflammation progresses. Table 1 gives the average composition of lymph.

Table 1: The average composition of lymph.

Compound	Concentration [g/L]
Sodium chloride	8.0
Glucose	0.7 – 1.0
Glycerol	1.0-10.0*
Triglycerides	1.5 – 13.2
Proteins (mostly albumin)	20- 25
Leukocytes	up to 500*

The influence of low molecular components capable of penetrating the carrier (a counter-current flow) and a substance capable of accumulating on the polymeric carrier surface were determined. In the first case the influence of salt (NaCl) present in physiological fluid, glucose and glycerol was determined; in the second protein (albumin) and triglycerides. Because of the specificity of the study, leukocyte accumulation was not tested. The model compound released from the carrier was cyanocobalamin.

2 MATERIALS AND METHODS

2.1 Materials

- cyanocobalamin (vitamin B12), Lowry Reagent, Folin & Ciocalteus Phenol Reagent, Sigma Aldrich
- NaCl, Beef albumin, glycerol, ethanol, POCh
- Glucose, Chempur
- Liquick Cor-TG 30 kit, Pz Cormay
- a module (Photo 1) containing asymmetric capillary tubes made of polysulfone with a 10kDa cutoff point (IBIB-PAN, Warsaw). The total geometrical area of the capillaries was $2.50 \cdot 10^{-3} m^2$.

Photo 1: The module with asymmetric capillaries made of polysulfone with a 10 kDa cut-off point (IBIB-PAN, Warsaw).

The solution of the model compound was pressed into the capillary, which was then circulated using a pump (Cole-Partner). On the outside of the capillaries, the receiver phase was circulating. The volumes of the phases were chosen to allow analysis of the model compound. The equipment was presented in Photo 2.

Photo 2: The equipment used in the study.

2.2 Determination of Cyanocobalamin Concentration

Cyanocobalamin concentration was determined spectrophotometrically at wavelength λ = 361 nm. Solutions of substance ranging from 0.005 g/L to 0.09 g/L were prepared and the standard curve: $Abs(361)=21.53 \cdot C$ [g/L] on the basis of the absorbance values was obtained.

2.3 Determination of Sodium Chloride Concentration

Sodium chloride concentration was measured by conductivity using a calibrated ionoselective

electrode. Solutions of substance ranging from 0.01 g/L to 1 g/L were prepared and based on the obtained conductivity values a standard curve was obtained: Conductivity [μS/cm] = 2198 · C [g/L].

2.4 Determination of Glucose Concentration – DNS Test

Glucose levels were determined by the DNS test (Miller, 1959). Solutions of substance ranging from 0.1 g/L to 1.9 g/L were prepared and the standard curve: Abs (550 nm) = 0.537 C [g /L] - 0.073 was obtained.

2.5 Determination of Glycerol Concentration

Glycerol concentration was determined by a Liquick Cor-TG kit. The analytical method is based on a colorimetric method with glycerolphosphate oxidase (Jacobs and Van Denmark, 1960). Glycerol solutions in the range of 0.1 g/L to 0.6 g/L were prepared and the standard curve: Abs (546 nm) = 1.30 · C [g/ L] + 0.266 was obtained.

2.6 Determination of Protein (Albumin) Concentration

Albumin concentration was determined by the Lowry method (Lowry et al., 1951). Albumin solutions were prepared at concentrations ranging from 0.02 g/L to 0.2 g/L. Based on the absorbance values obtained in the Lowry test, a standard curve: Abs (750 nm) = 3.39 · C [g/L] + 0.122 was obtained.

2.7 Mass Transport Study

Cyanocobalamin diffusion transport was investigated through polysulphone membrane. At the beginning of the study, the membrane was hydrophilized with 50% ethanol for 1 h, then the ethanol was washed with distilled water by continuously fed at 0.3 MPa (resulting in a permeate flow).

Modules used in the study (for each experiment a new one) contained 10 capillaries (length 0.071m, wall thickness 250μm, inner diameter 530μm). The total geometric area (an internal) of the capillaries was - $2.50 \cdot 10^{-3}$ m^2. The study was conducted at 37°C corresponding to the human body. In order to exclude the significant influence of the mass transport in continuous phases, they were subjected to circulation (in the range of 16-22 L/h). A cyanocobalamin aqueous solution at a concentration of 1 g/L (a model solution) was circulated on a tube side. The receiving solution was circulated on a shell side. The volumes of both phases were equal to 75 mL. The interface was maintained by establishing the flow of both phases and equalizing the pressure on both sides of the membrane.

In case of studying the effect of counter-current diffusion in the receiving phase in individual experiments, there were: NaCl at 8 g/L, 1g/L glucose or 10 g/L glycerol. The changes in the concentrations of the individual components were monitored in both phases at fixed intervals. All analysis was performed in duplicate.

Testing the influence of components (albumin, triglycerides) located on the membrane surface was preceded by sorption/deposition process. This process was performed at 37°C, for 2 h circulating on the outside of the membrane, the medium (75 mL) containing the tested components, which in individual experiments were triglycerides (at the concentration 10 g/L) or albumin (25 g/L). Inside the capillaries, water was circulated. The amount of substance adsorbed/ deposited on the surface of the membrane was calculated on the basis of the Lowry method for the protein and of the titrimetric method for triglycerides.

During the transport analysis, the sorbate was present still in receiving solution so that the sorption equilibrium was maintained throughout the whole process. The water present during the sorption process (circulating inside the capillaries) was replaced with a solution of 1 g/L cyanocobalamin.

The last stage of the study was to determine the transport of cyanocobalamin mass in conditions similar to those of living organisms. The research was preceded by the sorption of albumin and triglycerides, conducted at the circulation of the medium for 2h on the outside of the membrane at 37°C. The concentrations of the individual components in the circulation medium were 25 g/L albumin and 10 g/L triglycerides. Due to the addition of triglycerides the medium was emulsified. During the monitoring of the transport of cyanocobalamin mass, NaCl and glucose were added to the collecting medium to obtain a concentration of 8 g/L and 1 g/L, respectively. The concentration of cyanocobalamin in the intra-capillary circulating phase was 1 g/L and the flow of both phases was maintained at 16-22 L/h. Prior to spectrophotometric analysis, cyanocobalamin samples were centrifuged (10 min, 4000 rpm) to separate the fat phase.

3 RESULTS

Based on the changes of cyanocobalamin concentration, a mass flow was determined over time. Due to the continuous phase mixing, the constant concentration inside these phases was assumed, hence the mass flux density (n) was:

$$n = \frac{m}{A} = K \cdot \Delta C = K \cdot (C_{in} - \frac{C_{ex}}{P}) \quad (1)$$

By the mass balancing:

$$V_{ex} \cdot \frac{dC_{ex}}{dt} = K \cdot A \cdot \left(C_{in}(t) - \frac{V_{in}}{V_{ex} \cdot P}(C_{in,0} - C_{in})\right) \quad (2)$$

where: A – mass transport surface [m^2], C_{ex}, C_{in} - the concentration of the transported component in the receiving phase and inside the carrier, respectively [g/L], K – mass transport coefficient [m/s], m - mass flow [g/s], n - mass flux density [g/s·m^2], P – partition coefficient, P [-] = C^*_{in}/C^*_{ex} (C^* - equilibrium concentration [g/L]), t - time [s], V_{ex}, V_{in} - the volume of the receiving phase and the internal phase, respectively [L].

After integrating equation (2) with time from 0 to t we can obtain:

$$ln\frac{C_{in}(t=0)}{C_0 - \left(\frac{1}{P} + \frac{V_{ex}}{V_{in}}\right) \cdot C_{ex}} = \left(\frac{1}{P} + \frac{V_{ex}}{V_{in}}\right) \cdot \frac{K \cdot A}{V_{ex}} \cdot t \quad (3)$$

The linear dependence of $ln\frac{C_{in}(t=0)}{C_0 - \left(\frac{1}{P} + \frac{V_{ex}}{V_{in}}\right) \cdot C_{ex}}$ = f(t) confirms the correctness of the assumption of the flat concentration profile in continuous phases. Thus, mass transport coefficient can be easily determined from the equation (3) (Trusek-Holownia and Noworyta, 2003).

As the mass transport surface, the entire geometric surface of the membrane multiplied by a porosity factor (estimated from electron microscope images) at the level of 0.5 was taken. The possible error resulting from the estimation of porosity in relation to the calculations presented in the article is not significant, as the relative relations are analysed (exact the relations of mass transport coefficients determined in different conditions). Due to the environment within the capillaries and to the external environment, the value of the partition coefficient (P) was assumed to be 1.

3.1 Influence of Counter – Current Diffusion

The study was conducted for three low molecular weight substances found in the body fluids. A solution containing 8 g/L NaCl, 1 g/L glucose or 10 g/L glycerol was circulated on the receiving side. Changes in the concentration of the transported component (cyanocobalamin) were monitored in both phases until equilibrium was reached. An example of the change in cyanocobalamin concentration in the receiving phase for different media is shown in Fig. 1, while Table 2 shows the coefficients calculated from the concentration changes in both phases based on equation (3).

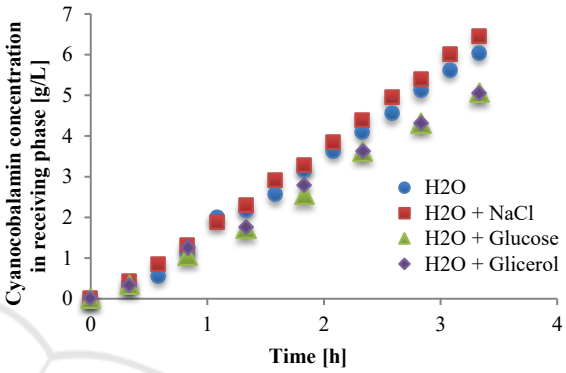

Figure 1: Cyanocobalamin concentration changes in time in receiving phase (water, water with NaCl at 8 g/L, waterwith glucose at 1 g/L and with glycerol at 10 g/L), 37°C, cut-off polysulfone membrane10 kDa, A=2.50·10^{-3} m^2.

Table 2: Mass transport coefficient for cyanocobalamin calculated on the base of equation (3), 37°C.

Receiving phase	K [m/s]	Error[m/s]
Water	3.34 ·10^{-8}	4.16·10^{-11}
Water + 8 g/L NaCl	3.96 ·10^{-8}	1.23·10^{-11}
Water + 1 g/L glucose	2.50 ·10^{-8}	5.70·10^{-11}
Water + 10 g/L glicerol	2.78 ·10^{-8}	3.22·10^{-11}

As expected, counter-current diffusers may affect the reduction of the mass transport coefficient. This phenomenon has been observed for organic compounds. The effect is especially visible for glucose, which was given at 10 times lower concentration than glycerol and the cyanocobalamin mass transport coefficient was reduced then by about 25%.

A different effect was observed in the presence of NaCl in the receiving phase. Salts of 10 g/L significantly increased the osmotic pressure and hence the effect of this phenomenon may be the reason for accelerated diffusion in the opposite direction. The mass transport coefficient increased in this case by 18.6%.

3.2 Effect of the Organic Layer on the Carrier Surface

Substances present in the body fluids may tend to settle on the surface of a polymeric carrier. This phenomenon will especially affect acute inflammation, where the leukocyte concentration is very high (up to 50% w/v in the lymph (Agrawal et al., 2008)). Due to the engineering nature of the study and the particular restrictions associated with leukocyte work, the impact of this component of the system was not investigated. The influence of the presence of protein (albumin) and triglycerides was tested. In the case of protein it is possible to talk about its sorption on the carrier and the sorption equilibrium present in the system. In the case of triglycerides, they are deposited on the surface.

Studies on the transport of cyanocobalamin mass were preceded by circulation of the protein solution at concentration (25 g/L) and a medium containing triglycerides (10 g/L). After two hours, the mass of the components on the membrane surface was estimated. They amounted to 11.4 g/cm^2 for the protein and 8.95 g/cm^2 for triglycerides. These values have not changed significantly (the range of analytical error) after leaving the system for another 22 h.

Table 3. Mass transport coefficient for cyanocobalamine for the carriers with and without organic compounds layer, 37ºC.

Receiving phase	K [m/s]	Error [m/s]
Carrier	$3.34 \cdot 10^{-8}$	$4.16 \cdot 10^{-11}$
Carrier with protein layer (11.4 mg/cm^2)	$3.14 \cdot 10^{-8}$	$4.75 \cdot 10^{-12}$
Carrier with triglyceride layer (8.95 mg/cm^2)	$3.04 \cdot 10^{-8}$	$4.67 \cdot 10^{-11}$

Table 3 lists the values of the mass transfer coefficient for cyanocobalamin for the carrier with and without the organic layer. Both in the presence of the protein layer and the triglycerides, the value of the mass transport coefficient has been reduced. However, it is not very significant (less than expected) decrease and does not exceed 6% lower of the value without any layer.

3.3 Transport in Multi-component System (Real Conditions)

In the last stage of the study, the carrier was covered with the layer of protein and triglycerides and cyanocobalamin was transported to the medium containing NaCl, glucose, glycerol, albumin and triglycerides at concentrations previously tested and corresponding to concentrations in the human fluids. Figure 2 shows the changes in cyanocobalamin concentration in the receiving phase and it was compared with process run for the carrier without layer with transport to water.

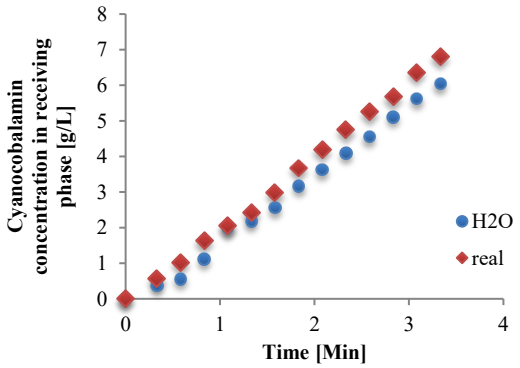

Figure 2: Cyanocobalamin concentration changes in time in receiving phase (water or in the medium simulating a body fluid) at 37ºC. Under real conditions the carrier was covered with protein and triglycerides layer.

The mass transport coefficient under real conditions was calculated on $7.78 \cdot 10^{-11}$ [m/s]. The value is 8.4% higher than this one obtained for carrier without layers and for water as receiving phase.

4 CONCLUSIONS

The studies indicate that the external environment should be considered for the precise design of drug carriers. Both high molecular weight substances, which are not capable of penetrating into the carrier, as well as the low molecular weight diffusers in the counter-current flow influence the mass transport coefficient from drug carriers.

Particular importance is the presence of salts in the body fluids. Probably, at their high concentration, the osmotic pressure generated by them promotes faster a mass release from carriers. As a result, this effect alleviates the opposite effects coming from compounds adsorbed on a carrier surface as well as the counter-current diffusion of low molecular organic compounds. The effect resulting from the presence of salt outside carriers can be minimized by using analogous salt concentrations inside carriers.

The present of leucocytes was not considered. It is expected that they can have a significant influence on mass release rate and this effect should be tested before medical therapies.

ACKNOWLEDGEMENTS

This work was performed within the Project No. 2013/11/B/ST8/03672 sponsored by the National Science Centre (NCN) of Poland.

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