

## **ANTICOAGULATION IN COMBINED MEMBRANE/ADSORPTION SYSTEMS**

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**Abstract:** For extracorporeal blood purification treatments, an effective anticoagulation is needed to avoid contact activation via the intrinsic pathway of the blood-clotting system.

While heparin is the standard anticoagulant in dialysis, it shows some disadvantages which have to be considered when it is used in membrane/adsorption-based blood purification systems. An alternative option for anticoagulation in these systems is citrate, which is effective as an anticoagulant by reducing the ionized calcium concentration in the extracorporeal circuit. However, to avoid citrate accumulation in the patient during treatment, the amount of citrate infusion and the citrate removal by the patient's metabolism as well as by dialysis have to be taken into consideration.

The aim of this study was to elucidate the characteristics of heparin removal in membrane/adsorption-based blood purification systems, to find the correct way to pre-coat adsorbents in order to avoid excessive adsorption of heparin by anionic exchanging resins, and also to find an appropriate dosage of heparin for treatments with these systems to ensure patient safety.

A further aim was to find the correct ionized calcium concentration to suppress complement activation, and to compare different dialysis filters regarding their citrate clearance in order to be able to recommend the correct dialysis setup to achieve appropriate citrate clearance.

We were able to show that the adsorptive removal of heparin can be significantly reduced by pre-coating the adsorbents with heparin without a perceptible impact on the adsorption kinetics of bilirubin. Furthermore, we recommend the use of unfractionated heparin due to its lower sieving coefficient and therefore lower removal compared to fractionated heparins.

Reducing the extracorporeal  $\text{Ca}^{2+}$  concentration to 0.2 mmol/L by infusion of citrate solution to the extracorporeal circuit results in an effective suppression of the complement activation.

To avoid citrate accumulation, we recommend the use of high flux filters when citrate anticoagulation is applied.

**Key words:** anticoagulation, citrate, heparin, liver support, adsorption, complement.

### *Introduction*

To avoid blood-clotting in the disposable system, extracorporeal blood purification systems (EBPS) need anticoagulation. In membrane-based EBPS such as haemodialysis or haemofiltration as well as in combinations of these procedures, unfractionated heparin (UFH) is widely used, especially in patients suffering from ESRD (end stage renal disease).

In acute patients who have more complex diseases such as acute renal failure, liver failure or sepsis, more complex EBPS are or will be clinically used. These systems are based on combined membrane/adsorbent technologies, which are able to eliminate protein/albumin-bound toxins, but also water-soluble substances, high molecular weight substances such as endotoxins (LPS), or even specific mediators such as TNF-alpha, IL 10 or complement factors (C5a).

Optimal anticoagulation for complex EBPS is one of the key factors which depend very much on the patient's situation (e.g. the level of AT III) and the characteristics of the membranes or adsorbents. Vice versa, the type of anticoagulation can influence the patient's reaction to the application of EBPS such as the level of  $\text{Ca}^{2+}$  concentration in citrate-anticoagulation to the activation of the complement system.

In our centre we investigated how to optimize the anticoagulation based on heparin or citrate-anticoagulation.

### *Materials and Methods*

#### *Heparin*

In our *in vitro* studies we compared the adsorption of heparins with different molecular mass, namely unfractionated heparin (UFH; Heparin Immuno<sup>®</sup> 5000 IU/ml, Baxter, IL, USA), Lovenox (Sanofi Aventis, Paris, France) and Arixtra<sup>®</sup> (= Fondaparinux; GlaxoSmithKline, London, UK) to polystyrene-divinylbenzene anionic exchange resins (PS-DVB) in fresh frozen plasma at an adsorbent concentration of 1:10 v/v. Furthermore, we evaluated the possibility of pre-coating these adsorbents with heparin and the effect of the coating on the adsorption kinetics for bilirubin and heparin. For the pre-coating, the adsorbents were incubated for 1 h in a sodium chloride solution containing 0, 10 and 100 IU heparin/ml. After the pre-coating procedure, the adsorbents were transferred

to a test tube with fresh frozen plasma (FFP) at a concentration of 1:10 (ratio adsorbent to total volume). To check the impact of heparinization on bilirubin adsorption, different amounts of bilirubin were added (5, 10 and 15 mg/dl). Heparin concentrations as well as bilirubin concentrations were measured in the supernatant at defined time points with a Hitachi/Roche 902 automatic analyzer using the photometric Choamatic Heparin test kit (Chromogenics, Epsom, UK) and the Bilirubin test kit (Roche Diagnostics, Mannheim, Germany), respectively.

Furthermore, we compared the applicability of different heparins for their use in membrane/adsorption-based blood purification systems regarding sieving coefficients and adsorption characteristics including high-flux dialysis but also the Albuflow<sup>®</sup> as one of the membrane filters of the Prometheus<sup>®</sup> liver support system.

#### *Citrate*

To find out the correlation between citrate concentration and the corresponding  $\text{Ca}^{2+}$  concentration, we added different concentrations of trisodium citrate solution (500 mmol/L, Mayrhofer Pharmazeutika, Austria) as well as ACD-A solution (113 mmol/L, Fresenius HemoCare, Germany) to freshly drawn blood and measured the ionized calcium and magnesium concentration with ion-selective electrodes (Nova 8 CRT, Nova Biomedical, MA, USA).

Another aim of our studies was to discover the correct citrate and the corresponding  $\text{Ca}^{2+}$  concentration to prevent activation of the complement cascade in the extracorporeal circuit. To check the impact on the suppression of the complement system, complement factor C3a was measured by ELISA (C3a-desArg, Progen Biotechnik, Germany) after activating the complement system by adding 5% v/v cellulose microparticles to freshly drawn blood for 15 min at 37 °C followed by adding different concentrations of trisodium citrate.

Furthermore, we investigated the influence of the patient's haematocrit (Hct) on the calcium reduction. This was carried out *in vitro* by adding defined amounts of trisodium citrate to fresh blood with different Hct which was prepared from blood drawn from one donor.

To check the removal rate for citrate by dialysis, clearance measurements were conducted by using an *in vitro* setup with the Fresenius 4008H dialysis machine and 1.2 L fresh frozen plasma at plasma and dialysate flow rates of 200 and 500 ml/min respectively. Citrate measurements were carried out by a photometric testkit (R-Biopharm AG, Darmstadt, Germany) on a Hitachi/Roche 902 automatic analyser.

The clearance measurements were carried out using polysulfone high flux and low flux filters with a 4008H dialysis machine (Fresenius Medical Care, Bad Homburg, Germany) and fresh frozen plasma at standard flow rates (200 ml/min and 500 ml/min for plasma and dialysate, respectively).

## Results

### Heparin

Figure 1 demonstrates the fast removal of heparins with different molecular weights in batch tests as well as in the whole system with an albumin filter. It clearly shows that the removal efficiency depends on the molar mass of the heparin: UFH (molar mass up to 25000) is eliminated more slowly than LMWH (Lovenox; molar mass 4000), while the synthetic heparin Arixtra® (Fondaparinux), which has a very low molar mass of 1728, shows the fastest removal.

Furthermore, we were able to show that, in the system set up with an albumin filter which separates the blood circuit from the adsorbent circuit, the difference in adsorption kinetics between UFH and LMWH is significantly higher than in batch tests.

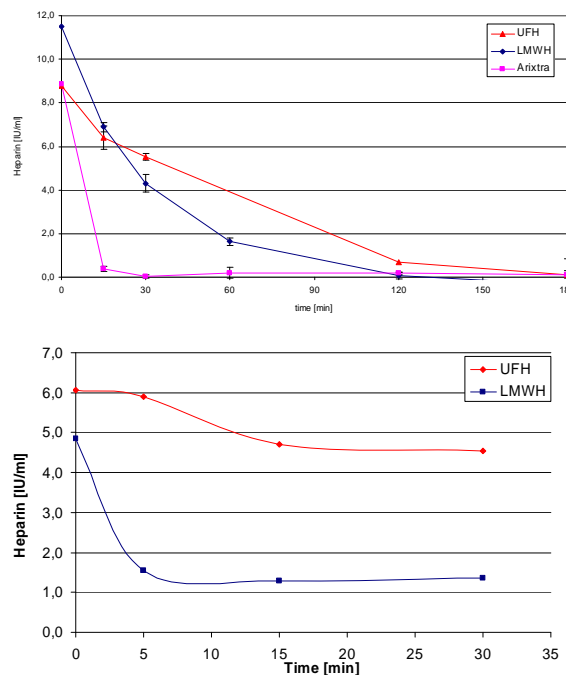


Figure 1 – Adsorption kinetics of heparins with different molar mass to anion exchanging PS-DVB in batch tests (left; adsorbent to plasma ratio = 1:9 v/v,  $n = 2$ ) and in the whole system incl. albumin filter (right; adsorbent to plasma ratio = 1:3.5 v/v).

Слика 1 – Кинетика на адсорпција на хепаринои со различна моларна маса низ анјон изменувачије PS-DBV во batch-тестови (лево; адсорбент во сооднос со плазма = 1:9 v/v,  $n = 2$ ) и низ целиот систем вклучувајќи го и албумин филтерот (десно; адсорбент во сооднос со плазма = 1:3.5 v/v).

Our results indicate that pre-coating of anionic PS-DVD resins leads to a reduction of the heparin adsorption during the treatment without a perceptible alteration of bilirubin adsorption kinetics. While coating with a heparin concentration of 10 IU/ml in the supernatant has a strong impact on the adsorption kinetics of heparin, only a small additional effect could be shown with heparin concentrations of 100 IU/ml. Furthermore, there was no significant effect on heparin adsorption when bilirubin was added at concentrations of 5, 10 and 15 mg/dl (Figure 2).

However, very high heparin amounts during the pre-coating procedure can slightly reduce the bilirubin-adsorption capacity. Figure 3 shows the bilirubin-adsorption kinetics for 3 different bilirubin concentrations (5, 10, 15 mg/dl) and 3 different pre-coating methods (0, 10, 100 IU/ml heparin in the supernatant during pre-coating).

Since unfractionated heparin binds to different proteins in the patient's plasma (especially to factor Xa and thrombin), its sieving coefficient is lower than expected and in the range of 8–12%. In contrast, from our own studies (unpublished data) it can be assumed that fractionated heparins have much higher sieving coefficients.

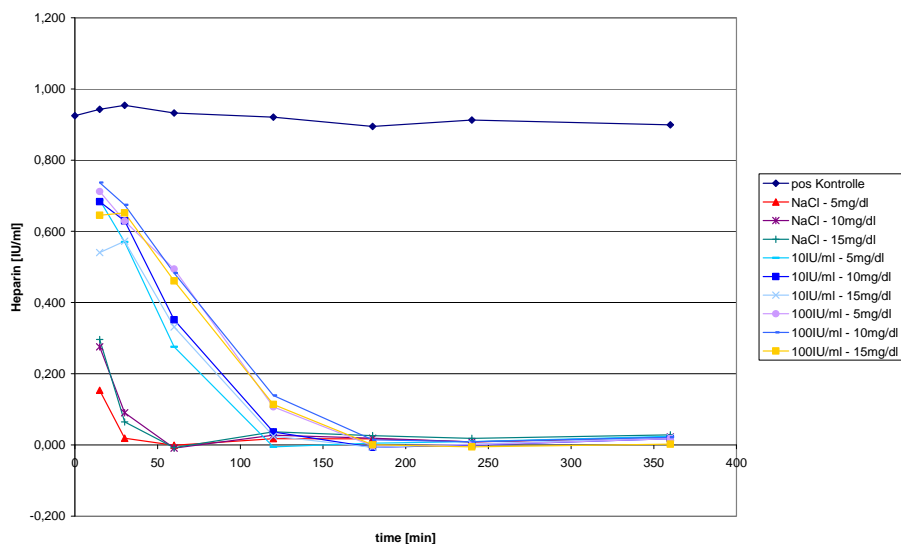


Figure 2 – Heparin adsorption of PS-DVB adsorbent after pre-coating with different amounts of heparin (batch-test)

Слика 2 – Адсорпција на хепарин од PS-DBV адсорбент  
по подготвување со различни количини на хепарин (batch-test)

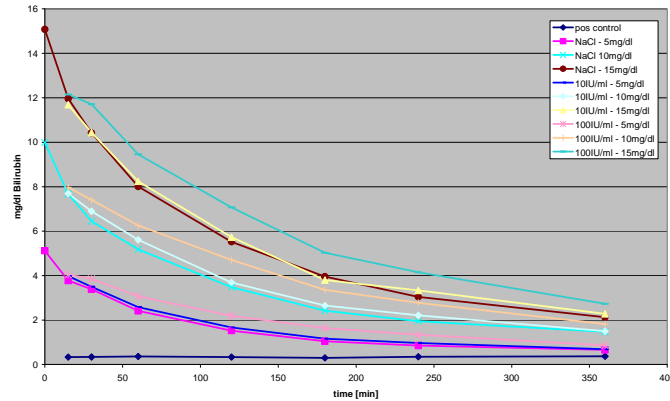


Figure 3 – Bilirubin adsorption of PS-DVB adsorbent after pre-coating with different amounts of heparin (batch-test)

Слика 3 – Адсорпција на билирубин од PS-DBV адсорбенту по подгојивување со различни количини на хепарин (batch-test)

### Citrate

The reduction of the ionized Calcium by different concentrations of citrate is shown in table 1.

Table 1 – Табела 1

Citrate concentration and the corresponding  $Ca^{2+}$  concentration in fresh blood from healthy donors.  $n = 10$

Концентрацијата на цитратот и одговарачката концентрација на јонскиот калциум во свежа крв од здрави донори

Citrate [mmol/L]	0	1	2	3	4	5	6
$Ca^{2+}$ [mmol/L]	$1.24 \pm 0.01$	$0.79 \pm 0.01$	$0.52 \pm 0.01$	$0.36 \pm 0.01$	$0.27 \pm 0.01$	$0.22 \pm 0.01$	$0.18 \pm 0.01$

Figure 4 shows the impact of citrate on the reduction of complement factor C3a. For an effective reduction of factor C3a, a  $Ca^{2+}$  concentration of 0.2 or slightly below 0.2 mmol/L is mandatory. The corresponding citrate concentration is 5 to 6 mmol/L. However, the amount of citrate needed to achieve a defined  $Ca^{2+}$  concentration strongly depends on the patient's Hct, since citrate distributes mainly in plasma [Whitfield 1981]. This is shown in Figure 5. At higher haematocrit, less citrate is needed to achieve a defined  $Ca^{2+}$  concentration.

In our studies we were able to show that high flux filters show significantly higher clearances than low flux filters ( $C = 114.2 \pm 2.6$  and  $C = 87.9 \pm 6.1$  ml/min·m<sup>2</sup>, respectively).

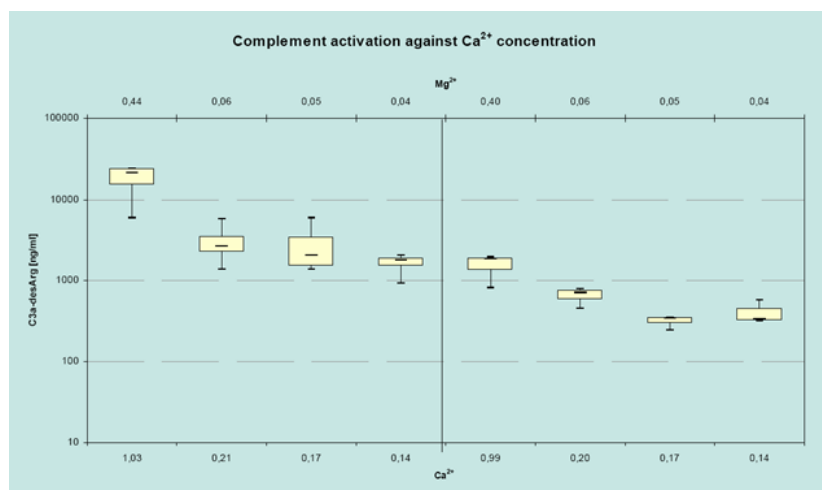


Figure 4 – Suppression of the complement activation (factor C3a) by reducing  $Mg^{2+}$  and  $Ca^{2+}$  with citrate. Left: blood activated with 5 % v/v cellulose microparticles. Right: control without cellulose. [Hartmann 2006]

Слика 4 – Намалување на актиџацијата на комплементот (фактор C3a) со редуирање на нивоата на јонскиот магнезиум и калциум со цитратот. Лево: крв актиџирана со 5 % v/v микрочестички од целулоза. Десно: контрола без целулоза [Hartmann 2006]

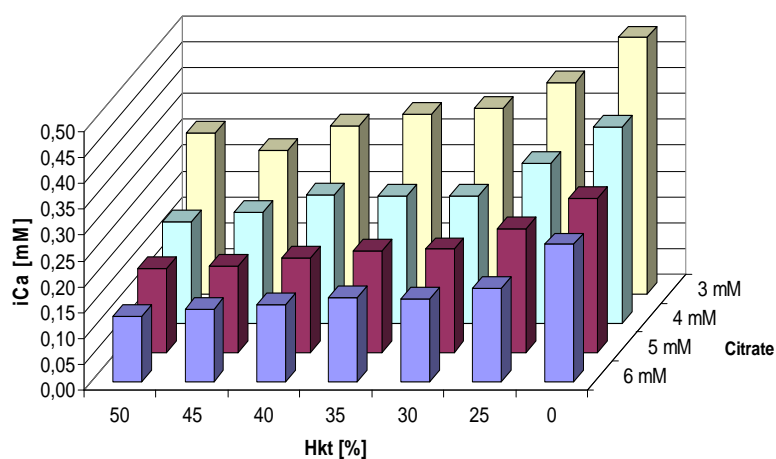


Figure 5 – Influence of the Hct on the  $Ca^{2+}$  reduction by citrate

Слика 5 – Влијанието на хематоцитот на намалување на нивоата на јонскиот калциум со цитрат

### *Discussion*

Since the removal rate of heparins is inversely proportional to their molar mass, it is strongly recommended to use UFH in membrane/adsorption-based blood purification systems. There are two reasons for the fast removal of smaller heparins. On the one hand, since the used adsorbents are highly porous substances with different pore sizes, the total accessible surface of the adsorbents is higher for small heparin molecules than for larger ones. Furthermore, smaller heparins have a higher sieving coefficient, which means a higher transfer rate of small heparins into the secondary circuit where the adsorption takes place. This is the explanation why the difference between UFH and LMWH removal was higher in the system experiments, where the albumin filter was used, than in the batch tests where the adsorbents were brought in direct contact with the heparin.

The reason for the relatively low sieving coefficient of UFH (8–12 %) for the Albuflow<sup>®</sup> filter is obviously the fact that heparins bind to different protein fractions in blood, especially to AT III, thrombin and factor Xa, but also to Hageman factor (XIIa) and factor IXa and fibrinogen, resulting in protein complexes of higher total molecular weight.

When the adsorbent circuit is not sufficiently anticoagulated, the clotting system could be activated and thrombin would be generated in the secondary circuit, which, after backfiltration through the albumin/plasma filter, could form fibrin, leading to blood clotting in the venous line of the extracorporeal system. This effect should especially be expected in patients with low ATIII levels. In order to avoid a heparin-free adsorbent circuit in combined membrane/adsorption-based EBPS, the use of UFH should be managed by heparin preloading, especially when anionic exchanger-based adsorbents are used. Since UFH partly acts via activation of AT III, anticoagulation with UFH is less efficient at low AT III levels. Therefore, at AT III levels below 50%, citrate anticoagulation should be preferred.

When citrate anticoagulation is applied, ionized calcium concentrations of 0.2 mmol/l in the extracorporeal circuit should be targeted to achieve adequate suppression of the coagulation as well as the complement cascade. Since citrate is mainly distributed in plasma, the patient's Hct has to be considered when citrate anticoagulation is applied. For patients with lower Hct, more citrate is necessary to achieve the target concentration of calcium but, for safety reasons, citrate concentration in the blood should not be higher than 6 mmol/L.

Citrate can be effectively removed via dialysis. The total removal rate depends on the filter type used and the filter surface, as well as on the blood and dialysate flow rates. Under optimal conditions, which means a high dialysate to blood-flow ratio, the citrate clearance can reach up to 95% of the blood-flow rate.



Since the total amount of infused citrate depends on the blood-flow rate, a higher blood-flow rate yields to higher citrate infusion to the patient. To avoid citrate accumulation, which is existent at a ratio of total to ionized calcium of  $> 2.5$  [Meier-Kriesche 2001], we suggest avoiding high blood-flow rates of more than 300 ml/min in combination with citrate anticoagulation since, even in combination with an effective dialysis, the total amount of citrate which is infused into the patient is higher. Furthermore, some dialysis machines, especially those for acute dialysis treatment in the intensive care unit, are not able to provide high dialysate flow rates of several 100 ml/min.

Patients with a severe disturbance of liver function have to be especially closely monitored when citrate anticoagulation is applied, since their citrate metabolism is impaired, leading to a higher risk of citrate accumulation and metabolic disorders. On the other hand, in patients with intact liver function, citrate is metabolized about twice as fast, increasing the risk of metabolic alkalosis due to an increase of bicarbonate in the patient's blood [Kramer 2003, Apsner 1997]. To overcome this risk, it is recommended to use dialysis concentrate with lower bicarbonate concentration when citrate anticoagulation is used. Furthermore, when citrate is infused in the form of trisodium citrate, the patient could suffer from hypernatraemia. Special dialysis concentrates with lower sodium concentrations are recommended in combination with trisodium citrate anticoagulation to reduce the risk of hypernatraemia. Furthermore, ACD-A solution (acid citrate dextrose) can be used, in which trisodium citrate is partly substituted by citric acid, resulting in a lower amount of sodium infusion into the patient.

Although heparin anticoagulation has some disadvantages in combination with membrane/adsorption-based blood purification systems, in this study we were able to show that it can be safely applied when the characteristics of these blood purification systems regarding heparin removal are considered. In the case of HIT (heparin induced thrombocytopenia) or other contraindications for heparin anticoagulation, citrate is a very elegant and effective alternative, due especially to its additional ability to suppress complement activation.

Currently, in our centre an automated system for citrate anticoagulation is under development, which is controlled by a software algorithm based on the results of this study [Brandl 2007].

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## Резиме

**АНТИКОАГУЛАЦИЈАТА ВО КОМБИНИРАН  
МЕМБРАНА/АДСОРПЦИЈА СИСТЕМ****Хартманн Ј., Штробл К., Фалкенхаген Д.**

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При третман со екстракорпорално прочистување на крвта, за избегнување на активацијата на внатрешниот пат на коагулирање на крвта преку контакт, потребна е ефективна антикоагулација.

Хепаринот како стандарден антикоагулант во дијализниот процес истовремено покажува некои недостатоци кои треба да се разгледаат кога се користи при мембрана/адсорпција базирани системи на прочистување на крвта. Како алтернативен избор за антикоагулација во овие системи е цитратот, чија делотворност како антикоагулант е во намалување на нивоата на јонскиот калциум во екстракорпоралниот круг. Сепак, за да се избегне акумулацијата на цитратот во пациентот за време на третманот, потребно е да се разгледа количината на инфундираниот цитрат како и цитратот отстранет од пациентот со неговиот метаболизам и со процесот на дијализата.

Целта на оваа студија беше да се разјаснат карактеристиките на отстранувањето на хепаринот во мембрана/адсорпција базираниот систем на прочистување на крвта, да го пронајде правилниот начин за (обложување)

на адсорбентите за да се избегне преголема адсорпција на хепаринот од неоргански изменувачките смоли, и исто така да се изнајде соодветно дозирање со хепарин за третман со тие системи за да се обезбеди сигурност за пациентот.

Понатамошна цел беше да се изнајде правилно ниво на јонски калциум за да се супримира активацијата на комплементот и да се споредат различни дијализни филтри во поглед на нивниот клиренс на цитратот за да бидат во состојба да препорачаат точно поставување на дијализата за да се постигне погоден клиренс на цитратот.

Ние бевме во состојба да покажеме дека отстранувањето на хепаринот со адсорпција може да биде значајно редуцирано со обложување на адсорбентите со хепарин без значајно влијание врз кинетиката на адсорпцијата на билирубинот. Покрај тоа, ние препорачавме користење на нефракциониран хепарин согласно со неговиот помал коефициент на поминување низ отворите на мембраната и поради тоа помалото отстранување споредено со фракционираниот хепарин.

Намалувањето на нивото на екстракорпоралниот јонски калциум на 0.2 ммол/Л со инфузија на раствор на цитрат во екстракорпоралниот круг резултира со делотворно намалување на активацијата на комплементот.

При примена на цитрат како антикоагуланс, за избегнување на акумулацијата на цитратот, ние препорачавме употреба на филтри со голема пропустливост.

**Клучни зборови:** антикоагулација, цитрат, хепарин, хепатална поддршка, адсорпција, комплемент.

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