Evaluation of VACUETTE® CAT Serum Separator Clot Activator Tubes for Therapeutic Drug Monitoring (TDM) in Serum

Background:

Greiner-Bio-One, Austria has been selling plastic evacuated tubes (VACUETTE®) for venous blood collection since 1986.

VACUETTE® Separator Tubes incorporate an inert gel material into the blood collection tube. These gels have a controlled viscosity and a specific gravity intermediate to the serum and clot. During centrifugation, the gel material forms an impermeable barrier between the serum and clot.

Study Objective:

The aim of this study was to show the stability of various commonly monitored drugs in sera when stored in two VACUETTE® CAT Serum Separator Clot Activator tubes with different gel polymers.

Study design:

Two tube types were evaluated in this study:

Sample	Description	Draw Volume	Size
Sample A US P-Gel	456073 VACUETTE® CAT Serum Separator Clot Activator	5 ml	13x100
Sample B European Gel	456073 VACUETTE® CAT Serum Separator Clot Activator	5 ml	13x100

Blood was taken from 16 healthy donors not on any form of medication or therapeutic regimen. One of each sample tube A and B was taken from each donor. The tubes were gently inverted and allowed to clot for at least 30 min. All tubes were centrifuged at 2200g for 15 min at 20°C.

Different control material for Ethanol, N-Acetylprocainamide and Procainamide, TCAs as well as one multiconstituent commercial control material for all other TDMs (see table 1) was required for the preparation of 8 serum pools in total.

By combining the sera, four serum pools were prepared, one for each control. Each serum pool was split and spiked, one with low and one with high concentration of each control material. The terms “low and high” are not specifically defined because the lower and upper limits of the therapeutic range vary depending on the drug.

The control material was added to the serum pool which was then transferred into 16 pre-labelled test tubes (4 controls with 1 low and 1 high concentration of each sample tube type). Four tubes (1 low and 1 high concentration of each sample tube type) were analyzed at 0h, 4h, 24h and 48h, respectively. All tubes were measured in duplicate and stored at 4°C.

Apart from the TCAs, the analysis of the drugs was performed on a Dimension Vista (Siemens) with the accompanying reagents by means of either LOCI™-Technology (Luminescent Oxygen Channeling Immunoassay), nephelometry, photometry or V-Lyte® Multisensor Technology. The TCA analysis was carried out by HPLC D-7000 (Merck-Hitachi).

The following 21 drugs were evaluated:

Table 1:

Antiepileptics[1)]	Phenytoin
	Valproic acid
	Carbamazepine
Cardiac glycoside[1)]	Digoxin
Alkaloids[1)]	Theophylline
Antiarrythmics[1)]	Procainamide
	n-Acetylprocainamide
Antibiotics[1)]	Gentamycin
	Vancomycin
	Tobramycin
Barbiturate[1)]	Phenobarbital
Alcohol[1)]	Ethanol
Trace elements[1)]	Lithium
Analgesics/Antipyretics[1)]	Acetaminophen
	Salicylic acid
Tricyclic antidepressants[2)]	Nortriptyline
	Amitriptyline
	Doxepin
	N-Desmethyldoxepin
	Imipramine
	Desipramine

[1)]Siemens Dimension Vista [2)] HPLC

Results / Comments:

Comparing the two VACUETTE® CAT Serum Separator Clot Activator tubes tested, apart from the TCAs, the study revealed that the recovery values were within +/-20% for Ethanol, Gentamycin, Vancomycin, Digoxin, Carbamazepine, Valproic acid, Phenobarbital, Salicylic acid, Procainamide, N-Acetylprocainamide and Theophylline.

As indicated by the graphs below, minimal deviations found equally in both gels for Lithium, Tobramycin, Acetaminophen and Phenytoin might be primarily due to the imprecision of the method as well as imprecise pipetting.

It should be considered that Acetaminophen is completely absorbed from the gastrointestinal tract and has a half-life of approx. 2 – 4 hours. The biological half-life or elimination half-life of a substance is the time it takes for a substance to lose half of its pharmacologic activity. Therefore, a rapid decline of the recovery values until the first measurement was found. The half-life of Phenytoin depends on the dosage administered [(4)]. The dosage of Lithium (half-life of approx. 24 hours [(5)]) is individually adjusted to every patient. The half-life of Tobramycin lies in the range of 0.5-15 hours [(5)].

Amitriptyline, Nortriptyline, Imipramin, Desipramine and Doxepin belong to the tricyclic antidepressants. The TCAs Amitriptyline, Imipramin and Doxepin belong to the so-called parent substances, whereas Nortriptyline, Desipramine and N-Desmethyldoxepin are the active metabolites, respectively. The last metabolite is not used as medication but is, however, of analytical importance in the treatment with doxepin. The mechanism of the TCA is based on the inhibition of the intake of the neurotransmitters norepinephrine and serotonin into the synaptic gap.

The half-lives of the TCAs lie within 6-50 hours. The dosage of the medication is increased step by step until the patient responds to the treatment. Since the therapeutic effect of TCAs takes place two to four weeks later, control analyses are more often recommended at the beginning of the therapy in order to ensure optimal therapeutic success. The stability of TCA results as demonstrated in this study suggest doing an analysis within 48 hours after medication intake, a time frame sufficient to assure the quality of the analysis and avoid loss of concentration due to absorption of the drug by the gel.

Conclusion:

The stability of therapeutic drugs in serum stored in gel tubes has been widely investigated. The absorption of drugs into the gel is dependent upon several factors including the chemical nature of the gel and of the drug itself, time on the gel, temperature of storage and volume of sample.

The stability of the drug on the gel up to 48 hours was shown for 21 drugs tested. From these results, it can be concluded that both gel polymers used in VACUETTE® CAT Serum Separator Clot Activator tubes show substantially equivalent performance concerning the selected drugs and variation in storage time.

References:

(1) Dasgupta A., Yared M.A., Wells A., Time-dependent absorption of therapeutic drugs by the gel of the Greiner Vacuette blood collection tube. TherDrugMonit. (2000)

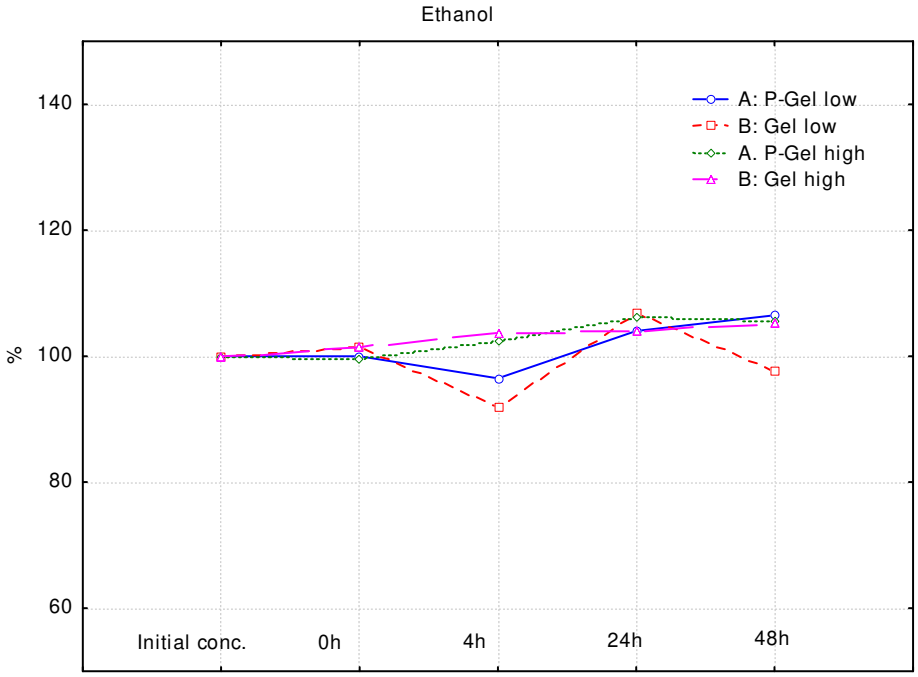
(2) Methods for the HPLC measurement – Benzodiazepine and Tricyclic Antidepressants in serum/plasma

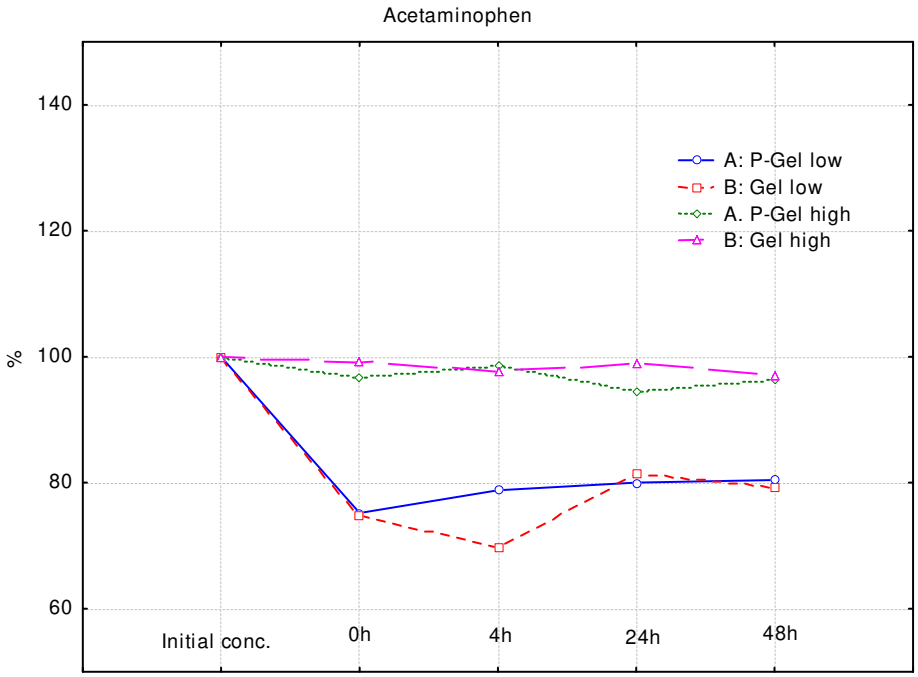
(3) Dimension Vista® System Flex® reagent cartridge – Reference for ACTM, DIGXN; Siemens Healthcare Diagnostics

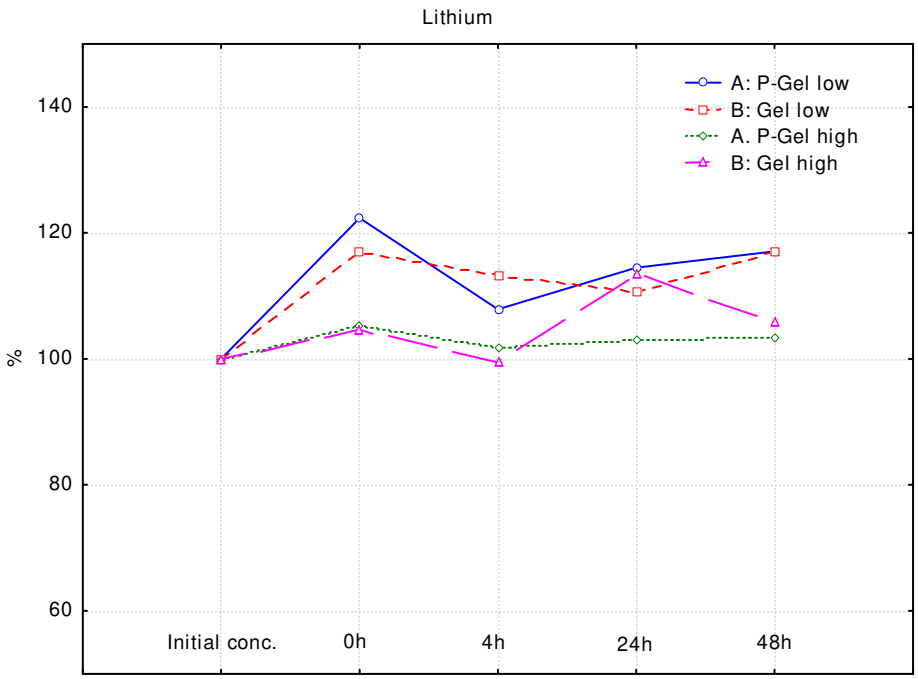
(4) Lothar T.: Labor und Diagnose – Indikation und Bewertung von Laborbefunden für die medizinische Diagnostik, 5. Aufl.; TH Verlagsgesellschaft mbH, Frankfurt am Main (1998)

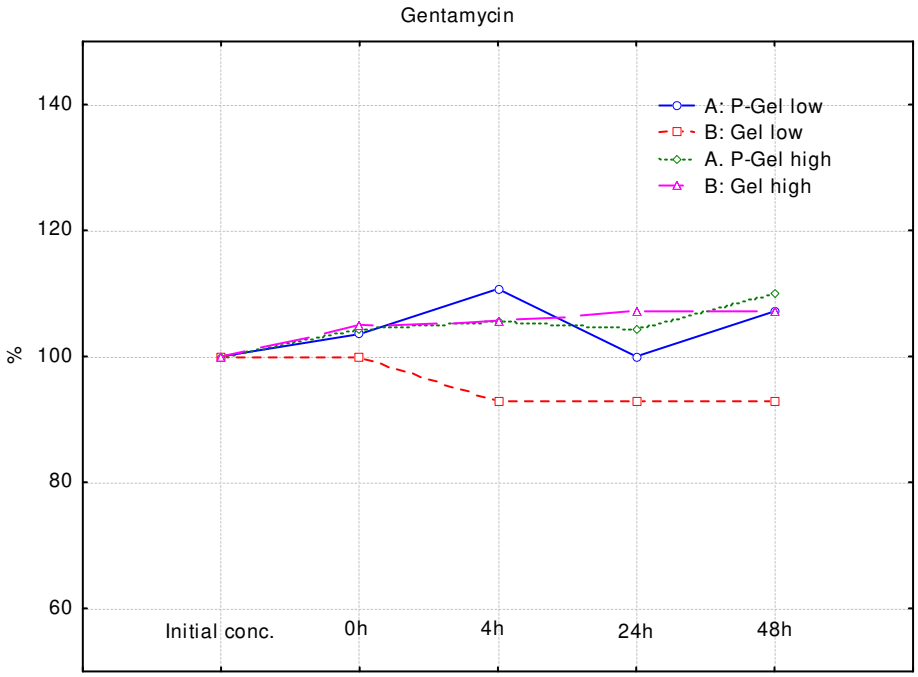
(5) Guder WG, Nolte J: Das Laborbuch für Klinik und Praxis, 2. Aufl. Urban & Fischer Verlag München (2009)

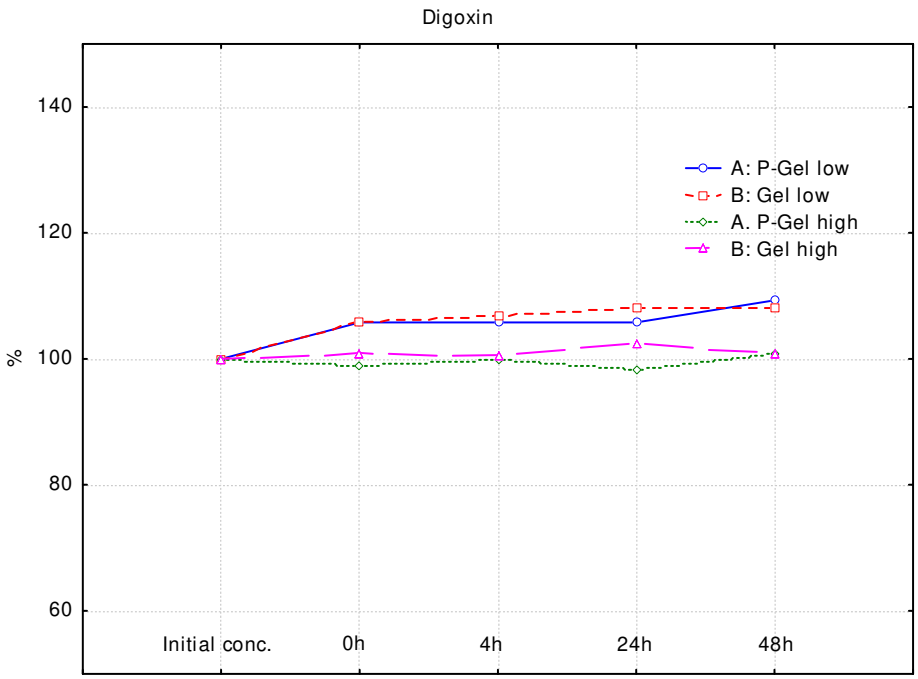
Results in detail:






Lithium
A: P-Gel low
B: Gel low
A. P-Gel high
B: Gel high
140
120
100
80
60
%
Initial conc.
0h
4h
24h
48h


Gentamycin
A: P-Gel low
B: Gel low
A. P-Gel high
B: Gel high
140
120
100
80
60
%
Initial conc.
0h
4h
24h
48h


Digoxin
140
120
100
80
60
%
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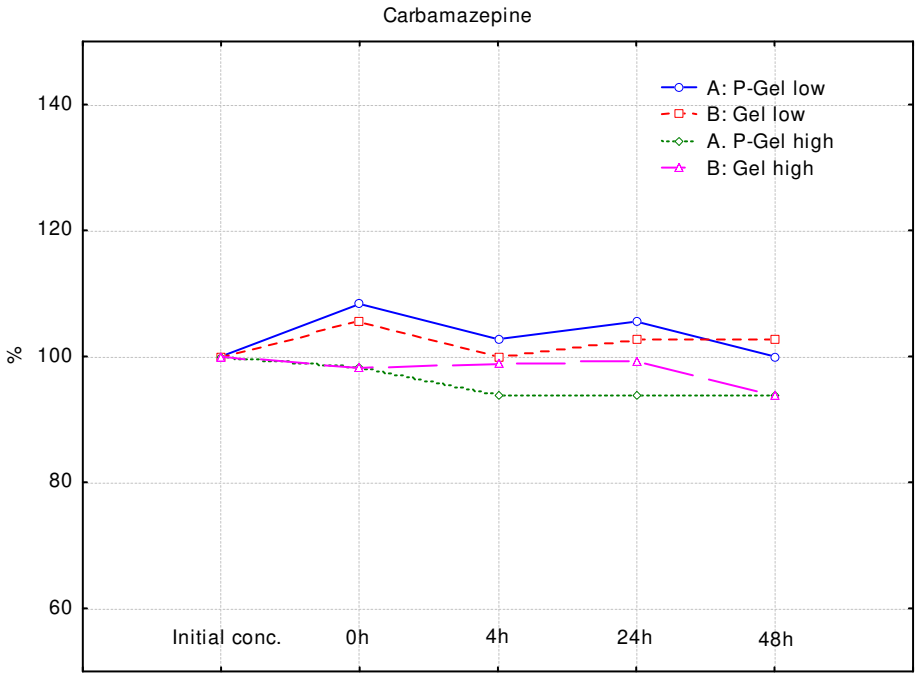
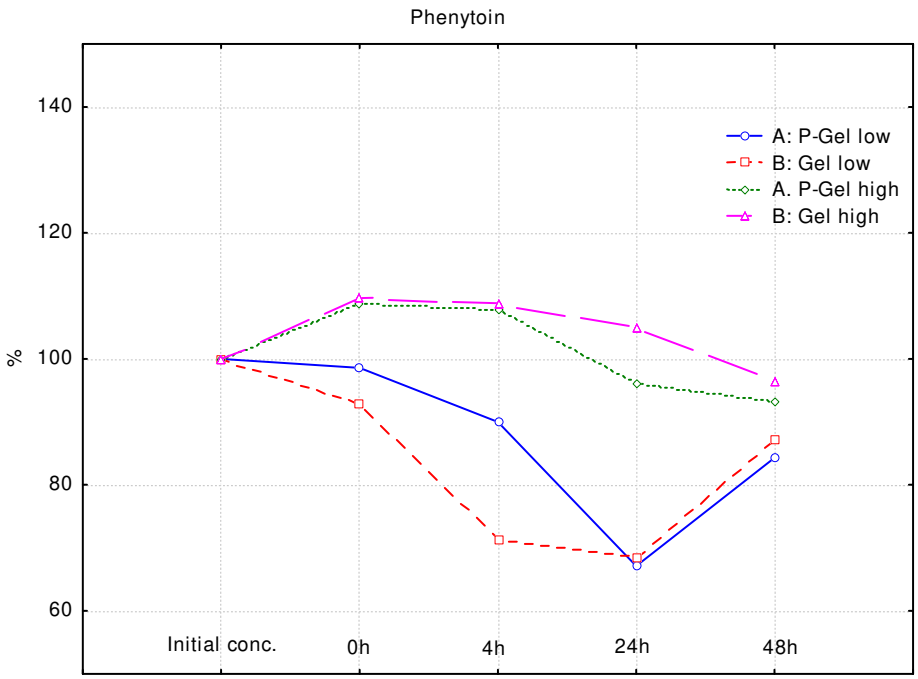
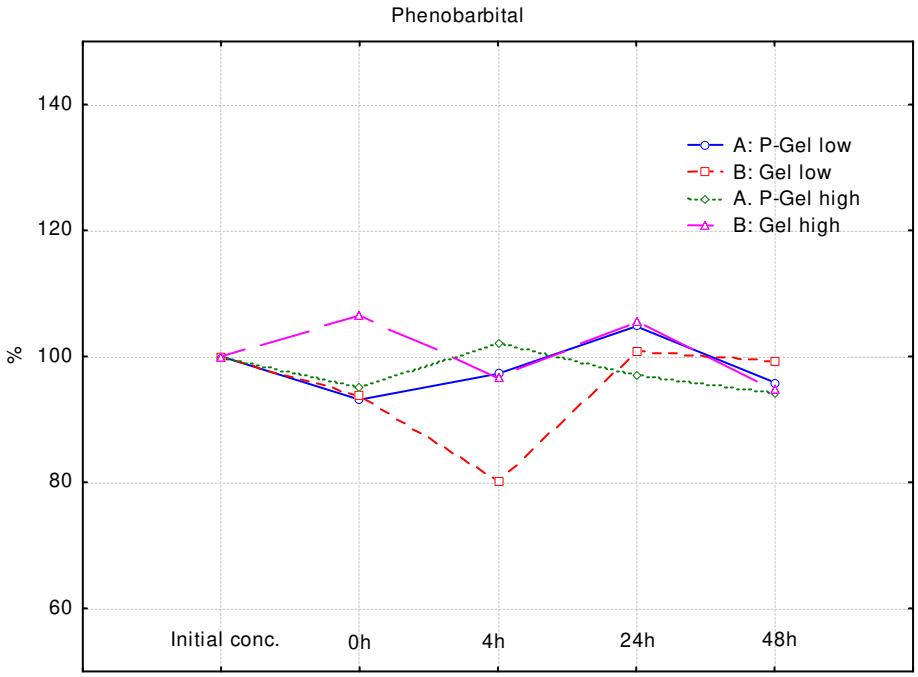
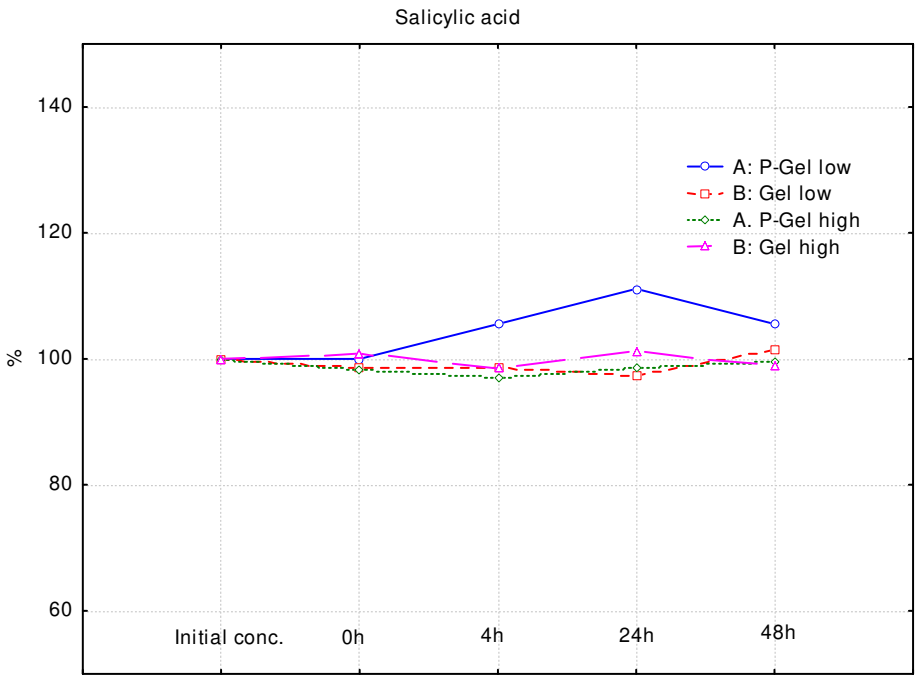
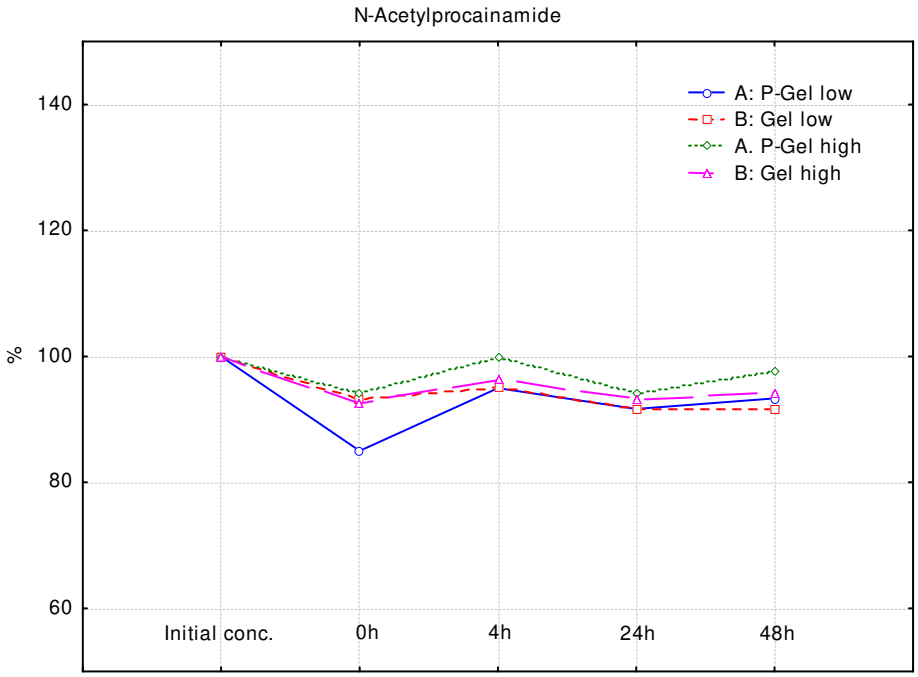
B: Gel low
A. P-Gel high
B: Gel high
Initial conc.
0h
4h
24h
48h

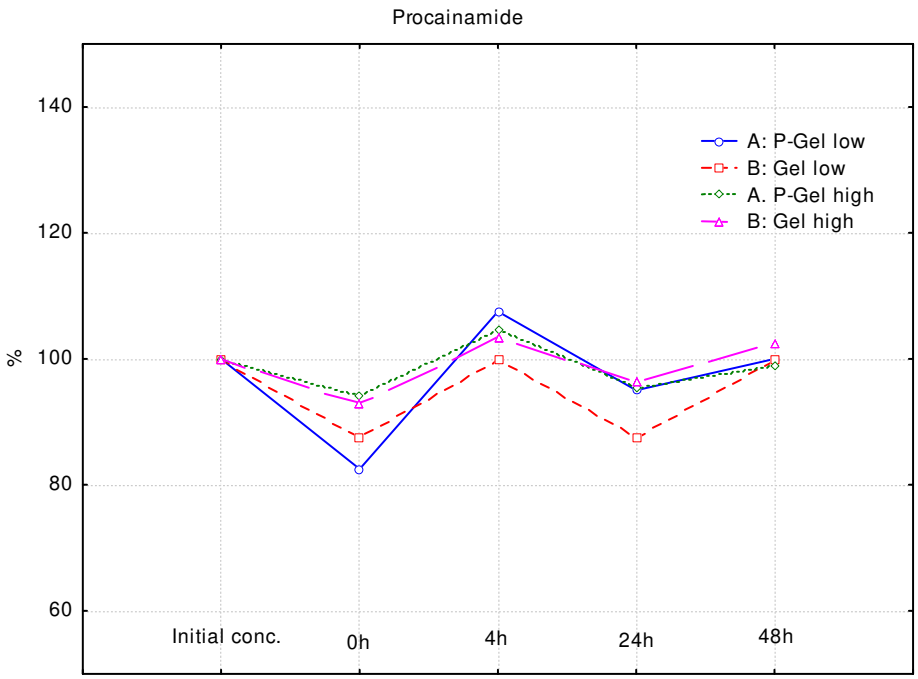

Carbamazepine
140
120
100
80
60
%
A: P-Gel low
B: Gel low
A. P-Gel high
B: Gel high
Initial conc.
0h
4h
24h
48h

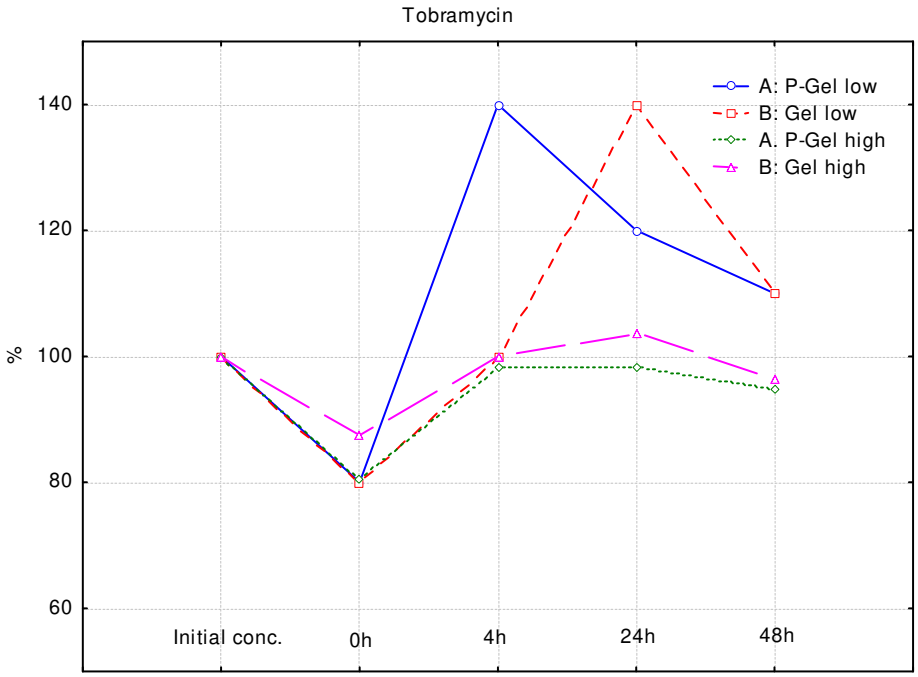

Phenytoin
140
120
100
80
60
%
A: P-Gel low
B: Gel low
A. P-Gel high
B: Gel high
Initial conc.
0h
4h
24h
48h

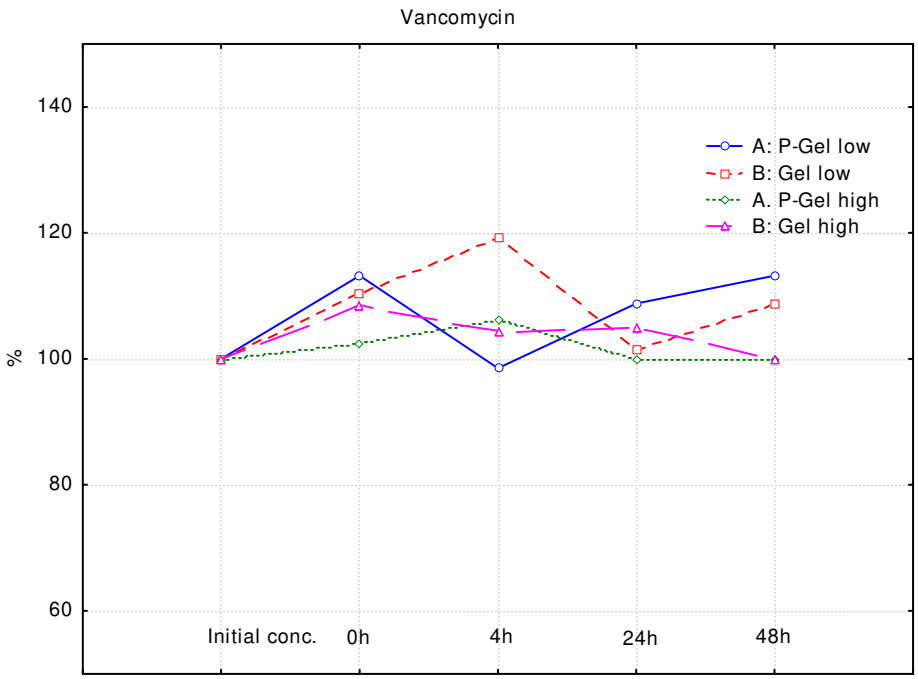

Phenobarbital
140
120
100
80
60
%
A: P-Gel low
B: Gel low
A. P-Gel high
B: Gel high
Initial conc.
0h
4h
24h
48h

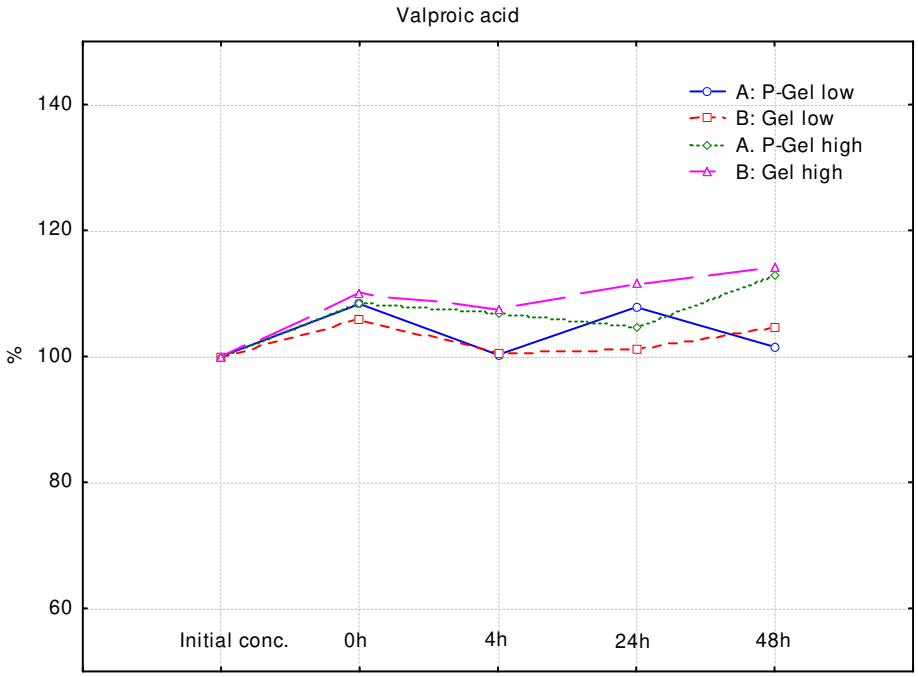

Salicylic acid
140
120
100
80
60
%
A: P-Gel low
B: Gel low
A. P-Gel high
B: Gel high
Initial conc.
0h
4h
24h
48h

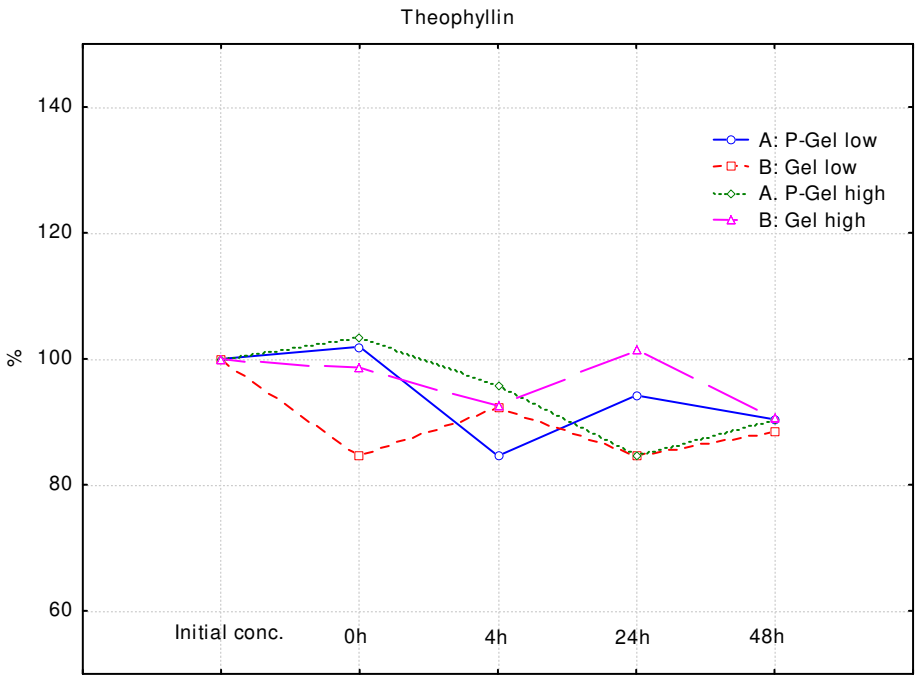

N-Acetylprocainamide
140
120
100
80
60
%
A: P-Gel low
B: Gel low
A. P-Gel high
B: Gel high
Initial conc.
0h
4h
24h
48h

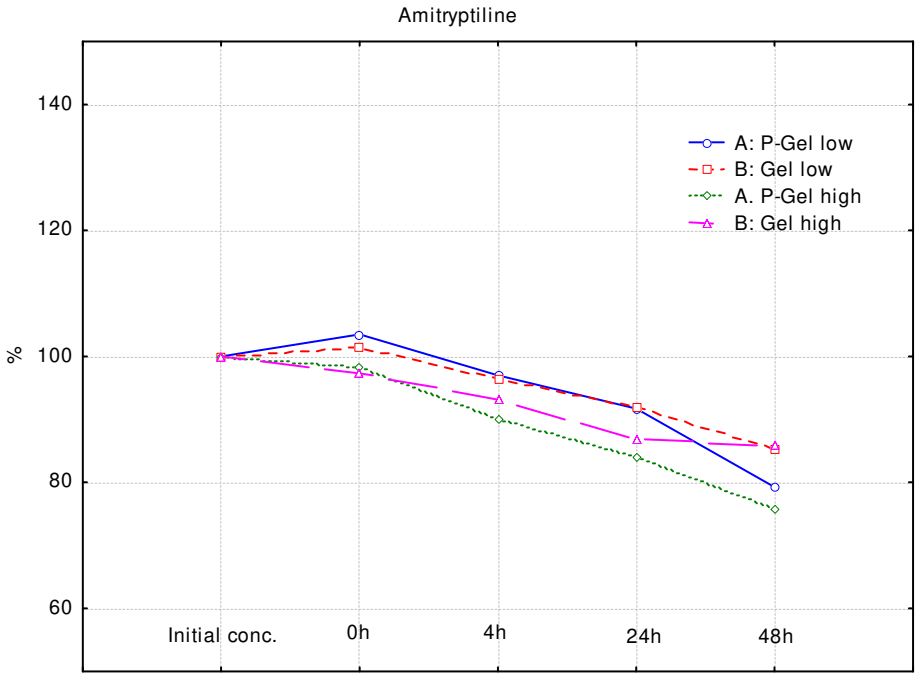

Procainamide
140
120
100
80
60
%
A: P-Gel low
B: Gel low
A. P-Gel high
B: Gel high
Initial conc.
0h
4h
24h
48h


Tobramycin
140
120
100
80
60
%
A: P-Gel low
B: Gel low
A. P-Gel high
B: Gel high
Initial conc.
0h
4h
24h
48h

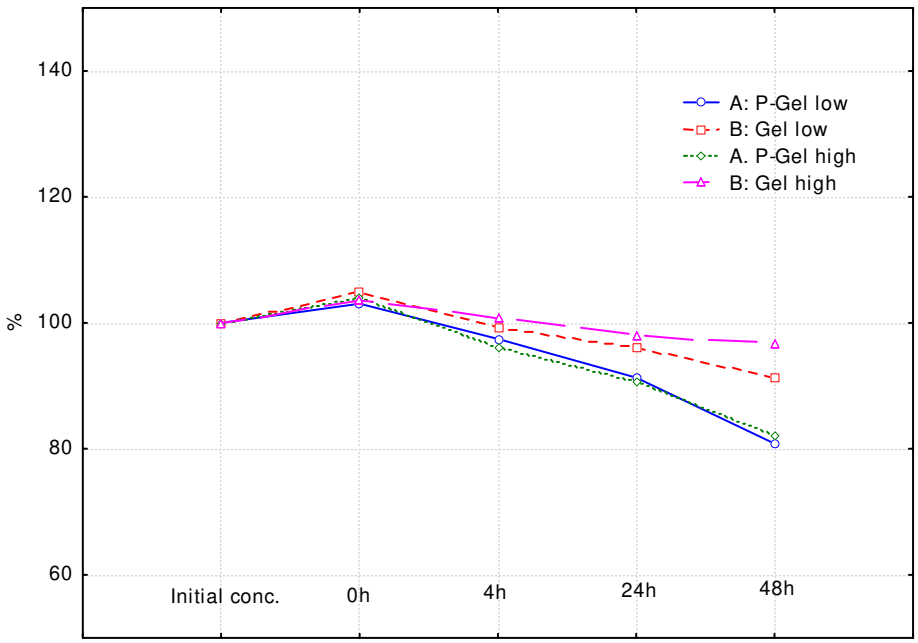

Vancomycin
140
120
100
80
60
%
A: P-Gel low
B: Gel low
A. P-Gel high
B: Gel high
Initial conc.
0h
4h
24h
48h


Valproic acid
140
120
100
80
60
%
A: P-Gel low
B: Gel low
A. P-Gel high
B: Gel high
Initial conc.
0h
4h
24h
48h

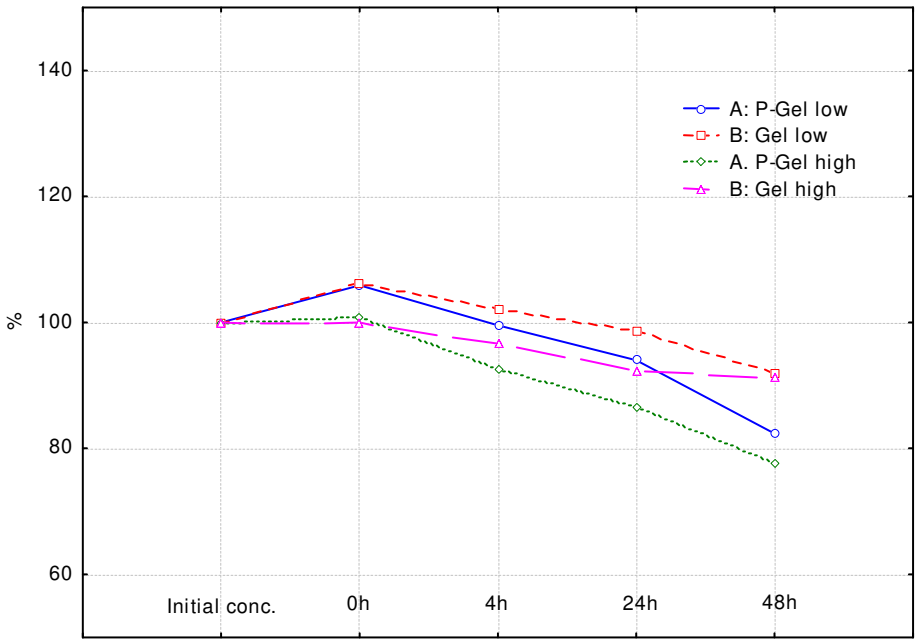

Theophyllin
140
120
100
80
60
%
A: P-Gel low
B: Gel low
A. P-Gel high
B: Gel high
Initial conc.
0h
4h
24h
48h

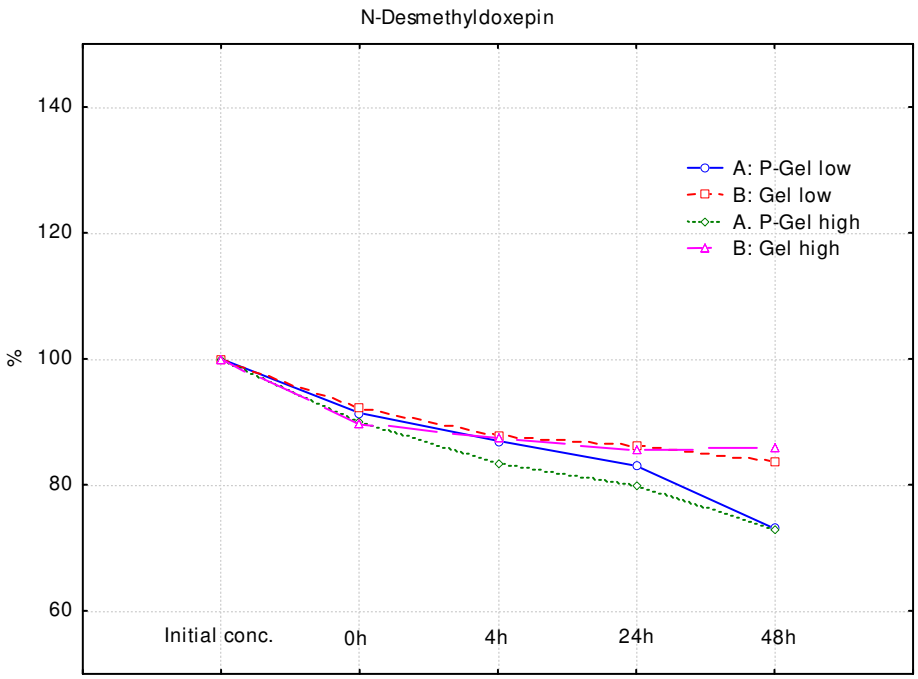

Amitryptiline
140
120
100
80
60
%
A: P-Gel low
B: Gel low
A. P-Gel high
B: Gel high
Initial conc.
0h
4h
24h
48h

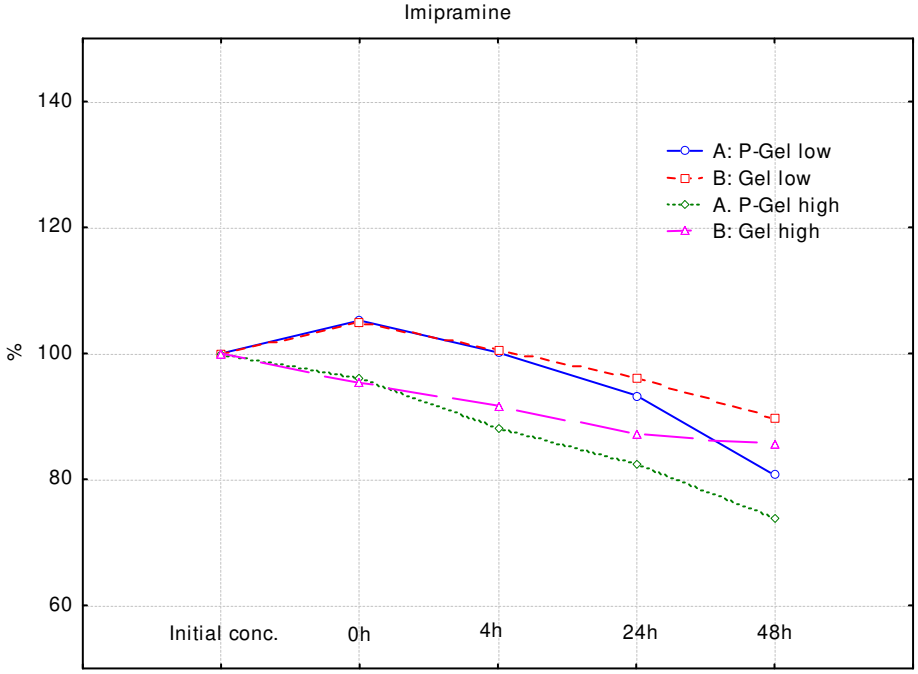
Nortryptiline

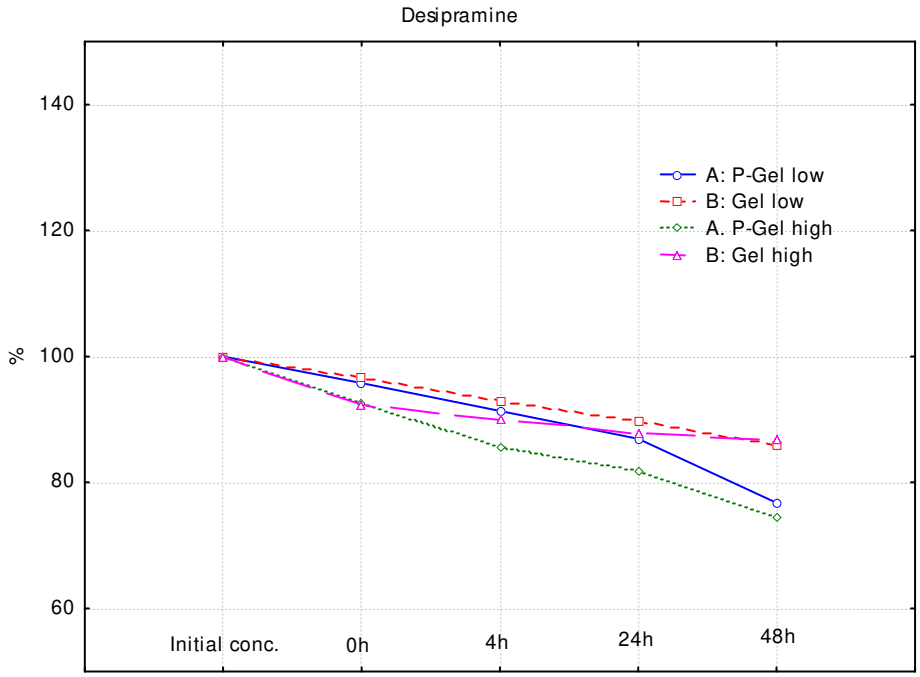


Doxepin




N-Desmethyldoxepin
140
120
100
80
60
%
A: P-Gel low
B: Gel low
A. P-Gel high
B: Gel high
Initial conc.
0h
4h
24h
48h


Imipramine
140
120
100
80
60
%
A: P-Gel low
B: Gel low
A. P-Gel high
B: Gel high
Initial conc.
0h
4h
24h
48h


Desipramine
140
120
100
80
60
%
A: P-Gel low
B: Gel low
A. P-Gel high
B: Gel high
Initial conc.
0h
4h
24h
48h