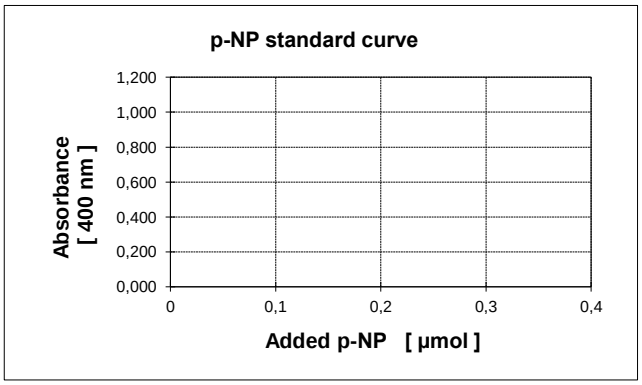
Name:
Date:

Experiment 3
Studies on phosphatase
Results

1. Preparation of p-NP reference curve

p-NP stock solution	0,1	(mmol/l)						
Standard curve:	t e s t t u b e s							
	Blank	1	2	3	4	5	6	7
0.1 mM p-NP-solution (ml)	0	0,2	0,5	1	1,5	2	2,5	3
Added p-NP (µmol)	0	0,02	0,05	0,1	0,15	0,2	0,25	0,3
A(400)								
A(400)-A(vak)								
tgα (slope):								

The Absorbance of 1 µmol p-NP at 400nm:



2. Substrate concentration dependence of the reaction time

	t e s t t u b e s							
	1	2	3	4	5	6	7	8
A-substrate-solution								
B-substrate-solution								
C-substrate-solution								
D-substrate-solution								

Test tubes	$(S)_{theor}$ (mmol/l)	(S)real (mmol/l)	A	A-Blank	p-NP (µmol)	v (nkatal)
1	0,375	0,125				
3	0,600	0,200				
5	1,000	0,333				
7	3,000	1,000				

[1mol/sec=1kat]
Reaction time: 900 sec

Test tubes	(S)r (mmol/l)	1/(S)r	v (nkatal)	1/v
1	0,125	8,000		
3	0,200	5,000		
5	0,333	3,000		
7	1,000	1,000		

4. Competitive inhibition with inorganic phosphate (0.1 mmol/l):

	test tubes							
	1	2	3	4	5	6	7	8
A-substrate-solution								
B-substrate-solution								
C-substrate-solution								
D-substrate-solution								

Test tubes	$(S)_{theor}$ (mmol/l)	(S)real (mmol/l)	A	A-Blank	p-NP (µmol)	v (nkatal)
1	0,375	0,125				
3	0,600	0,200				
5	1,000	0,333				
7	3,000	1,000				

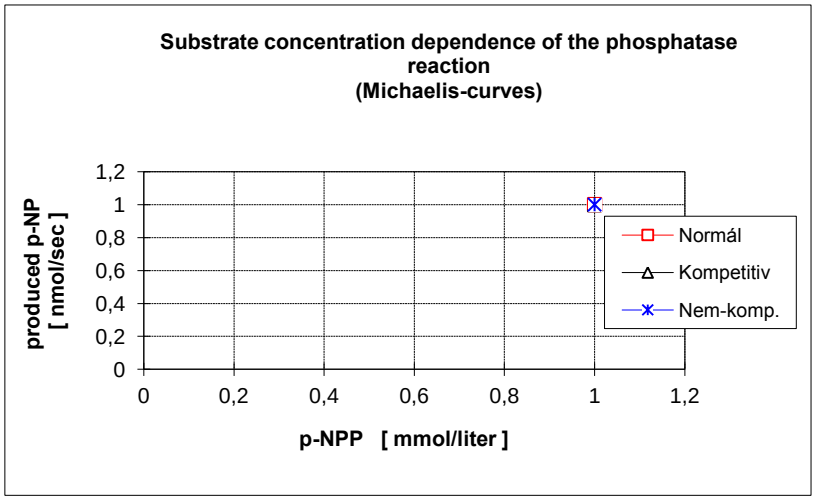
Test tubes	(S)r (mmol/l)	1/(S)r	v (nkatal)	1/v
1	0,125	8,000		
3	0,200	5,000		
5	0,333	3,000		
7	1,000	1,000		

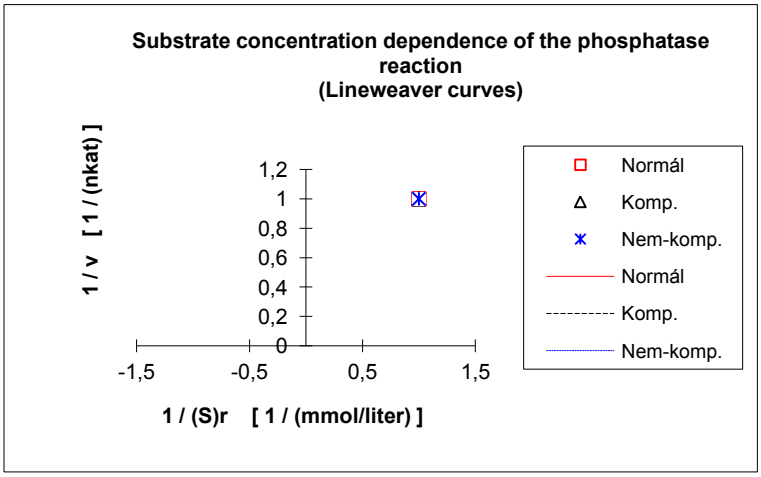
5. Non-competitive inhibition with NaF (0.1 mmol/l):

	test tubes							
	1	2	3	4	5	6	7	8
A-substrate-solution								
B-substrate-solution								
C-substrate-solution								
D-substrate-solution								

Test tubes	$(S)_{theor}$ (mmol/l)	(S)real (mmol/l)	A	A-Blank	p-NP (µmol)	v (nkatal)
1	0,375	0,125				
3	0,600	0,200				
5	1,000	0,333				
7	3,000	1,000				

Test tubes	(S)r (mmol/l)	1/(S)r	v (nkatal)	1/v
1	0,125	8,000		
3	0,200	5,000		
5	0,333	3,000		
7	1,000	1,000		





Discussion:

Normal reaction		
Km		**mmol/l**
Vmax		**nkat**
Competitive-inhibition		
Km		**mmol/l**
Vmax		**nkat**
Non-competitive inhibition		
Km		**mmol/l**
Vmax		**nkat**