

Biochemical and biophysical experimental methods in the study of dental allergy I&II parts: Biocompatibility of dental materials – Allergy – Molecular biology methods in allergy detection

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Molecular biology methods in dentistry – elective
course

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Possible dental allergens

- impression materials
- local anesthetics, medications
- disinfectants
- materials for dentures (alloys and polymers)
- restorative materials (filling materials)
- adhesive materials

Aim of the study

Period: between 1996 and 1998 in Hungary

N=147

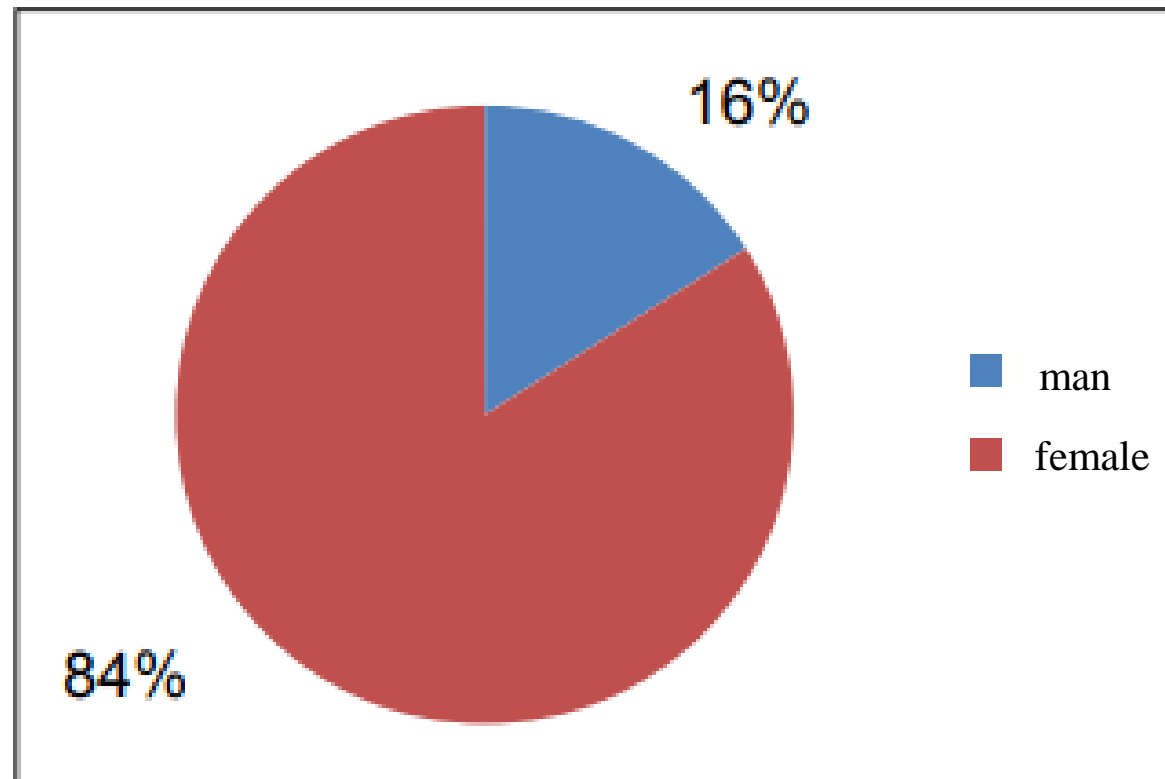
Place: DE FOK

Brial allergens (Breven, Germany)

- gender and age distribution
- To study frequency of dental allergens
- The allergological examination is performed according to the current Hungarian professional protocol
- Epicutaneous tests (4 days, reading days: Wednesday, Thursday, Friday)

Gender distribution in studied patient group

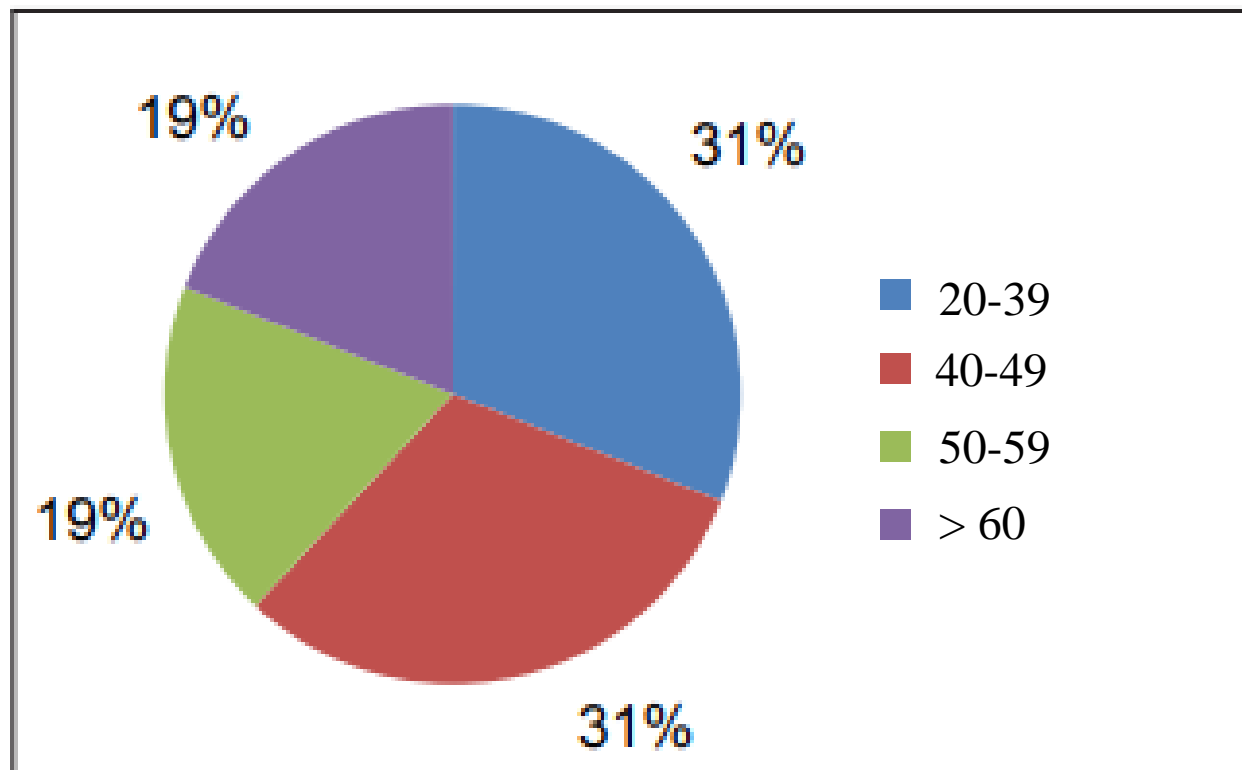
See Fig. 1 in Szepesi's et al article



female dominance

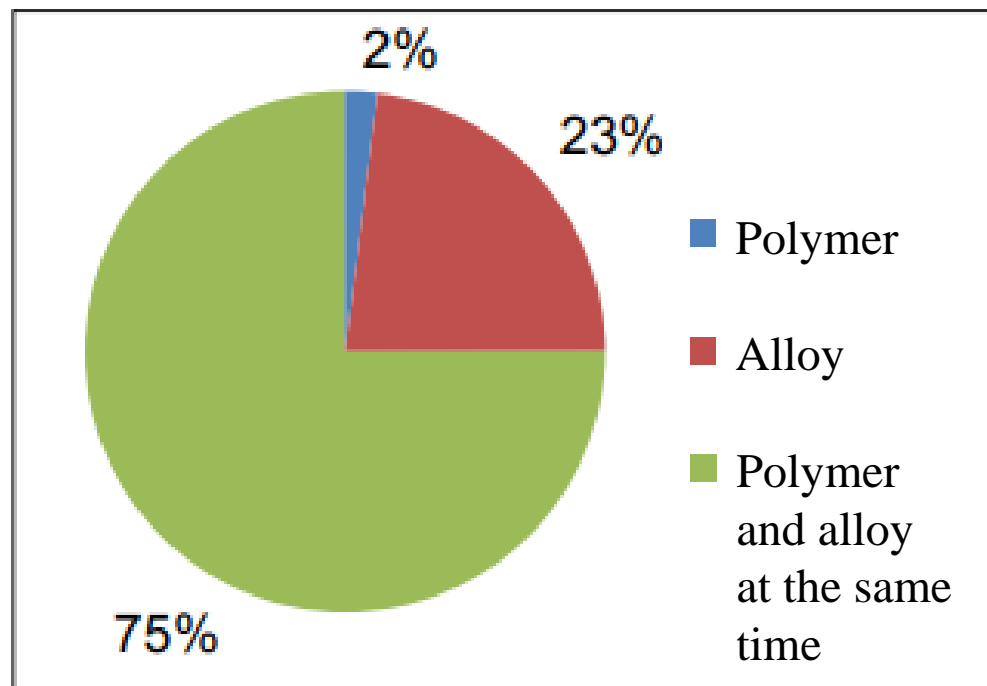
Age distribution in the studied patient group

See Fig. 2 in Szepesi's et al article



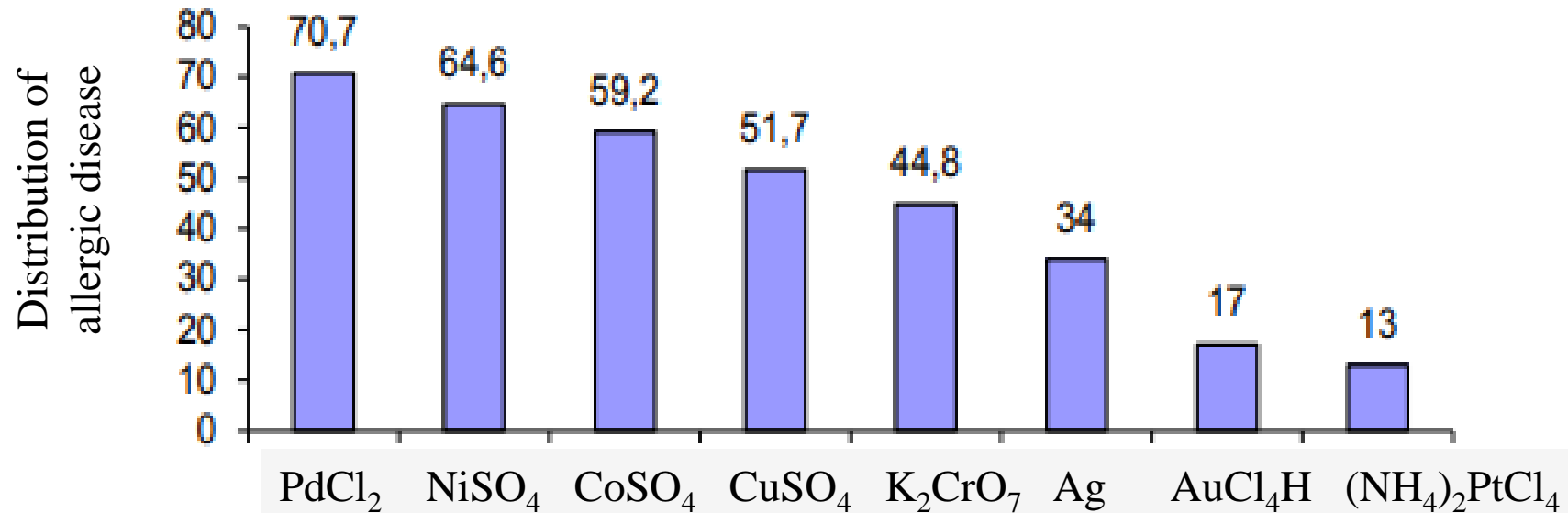
Hypersensitivity distribution of the studied patient groups according to the main groups

See Fig. 3 in Szepesi's et al article



Distribution of metal allergens in the studied patient group

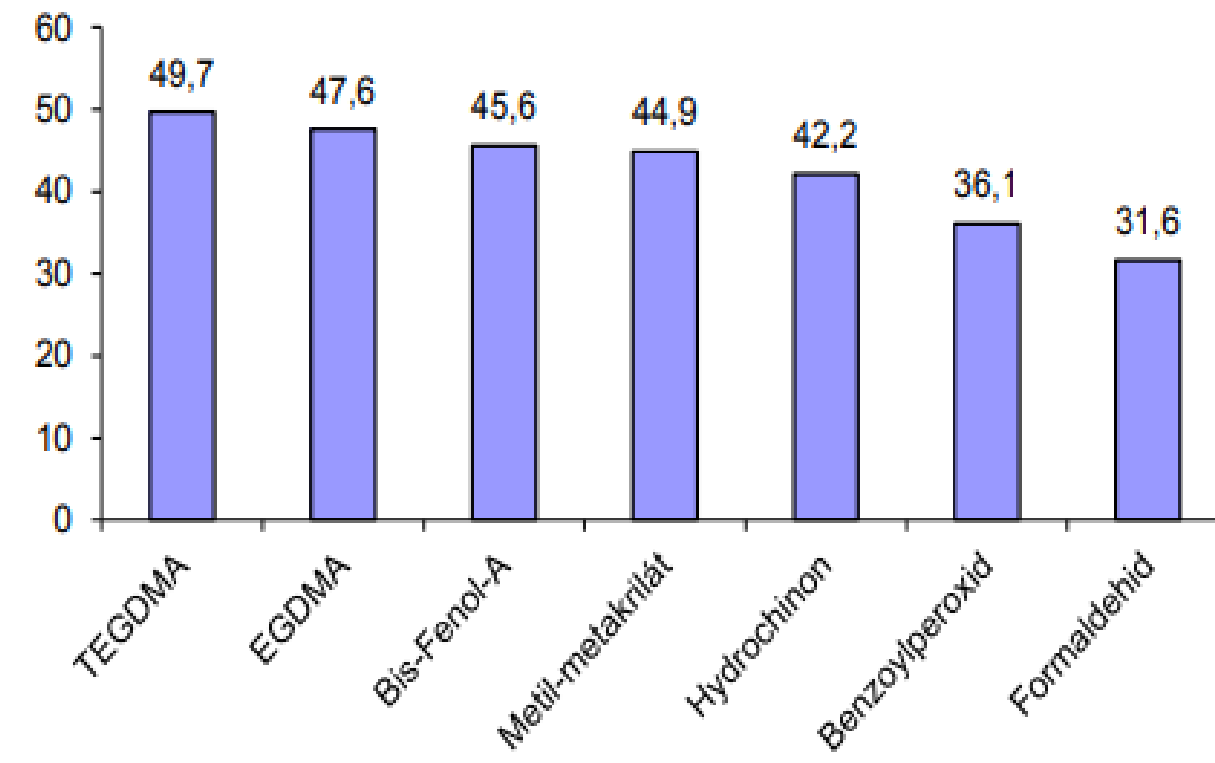
See Fig. 4 in Szepesi's et al article



Ni and Pd dominance

Distribution of polymer allergens in the studied patient group

See Fig. 5 in Szepesi's et al article



Methacrylate dominance

Conclusions

- The most frequent women and aged 20-49
- Among polymers the most frequent dental allergen: Triethylene glycol dimethacrylate (TEGDMA)
- Among the alloys the most frequent dental allergen: palladium chloride
- The most frequent form: combined allergy (alloy and polymer at the same time)

Connection between Salivary histatin-5 and Ni allergens

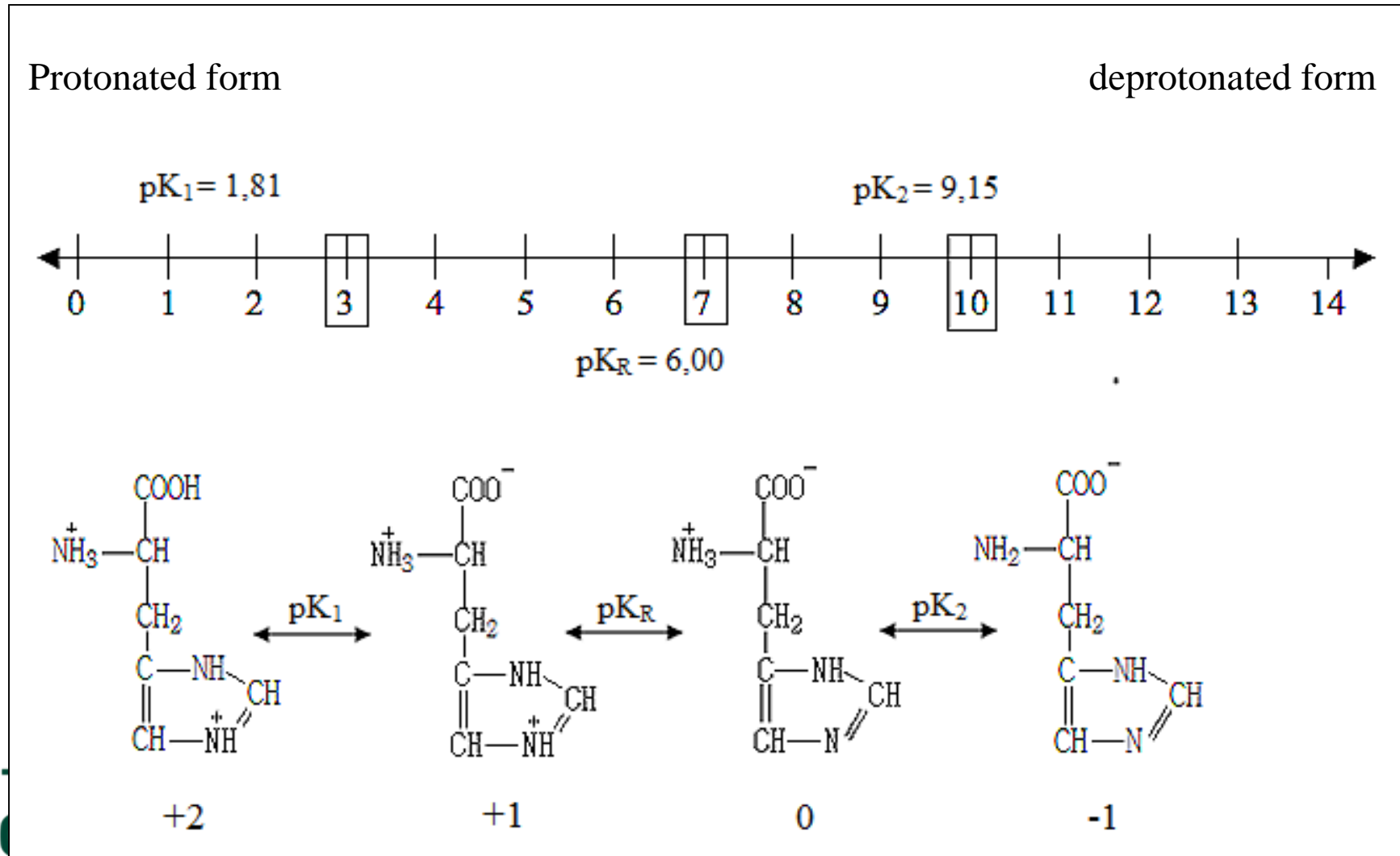
See Scheme 1 in
Kurowska's et al
article

Histatin in saliva is a low molecular weight, histidine (His) rich protein, which is primarily part of the submandibular salivary gland and parotid secretion, is part of innate immunity, and has a strong antibacterial, antifungal effect.

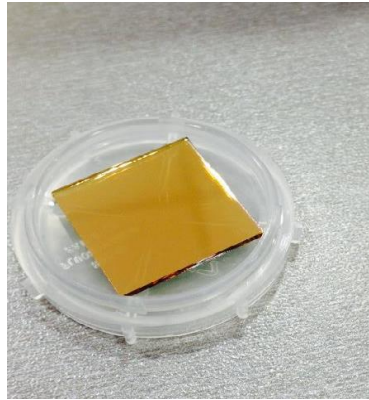
Histidine member

Helmerhorst, E.J.; Troxler, R.F.; Oppenheim F.G. (2001): The human salivary peptide histatin 5 exerts its antifungal activity through the formation of reactive oxygen species. PNAS 98: 14637-14642.

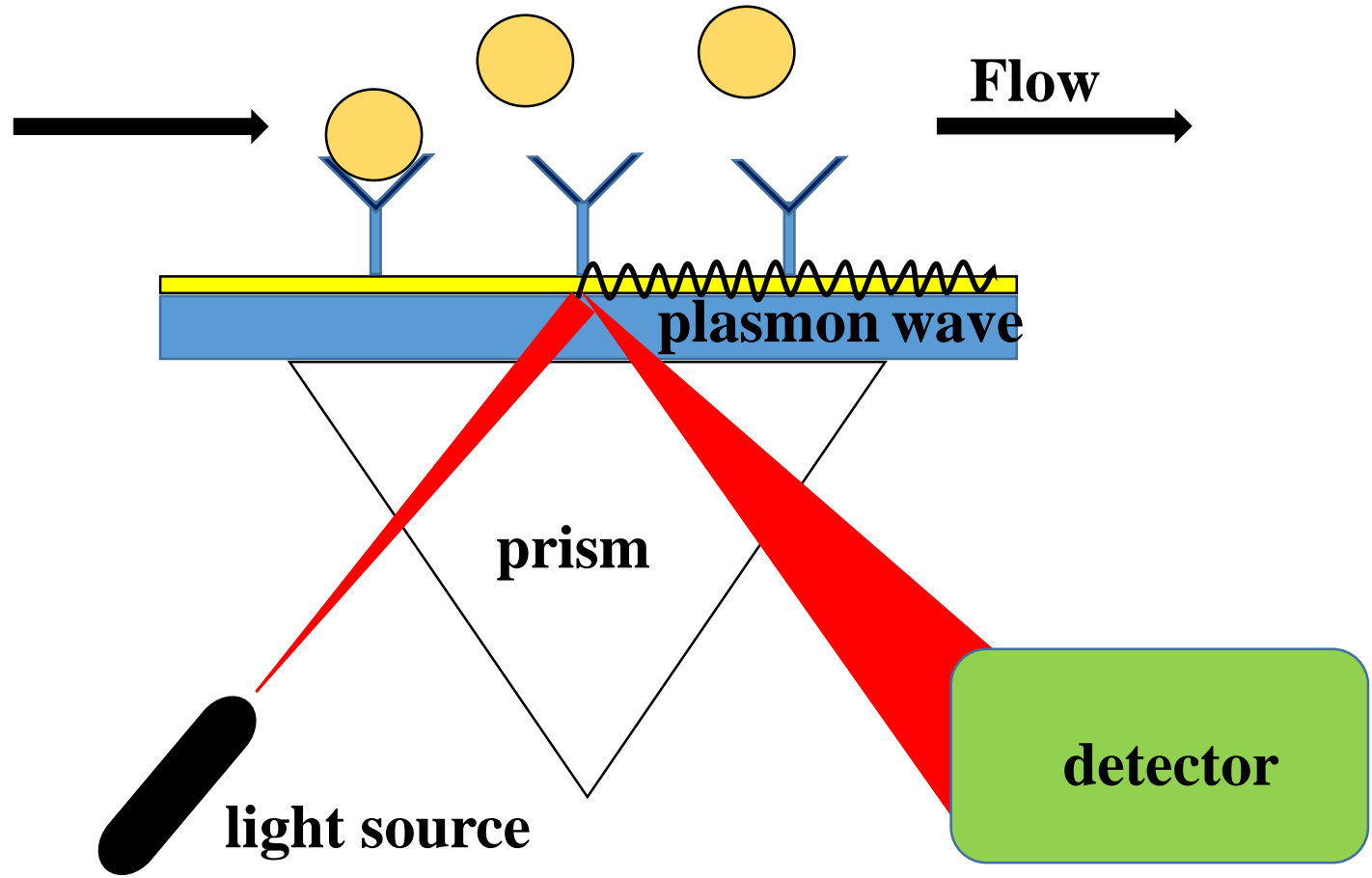
Molecular charge of Histidine depending on pH



Role of Surface Plasmon Resonance (SPR) spectroscopy in molecular binding detection

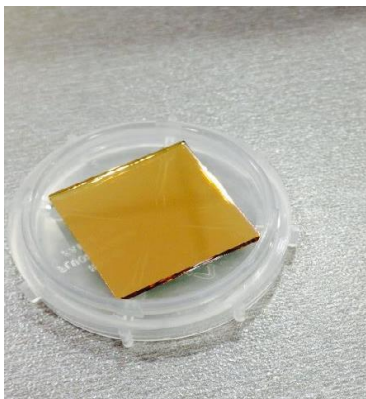


SPR chip: 45 nanometer thick thin layer of gold on glass surface

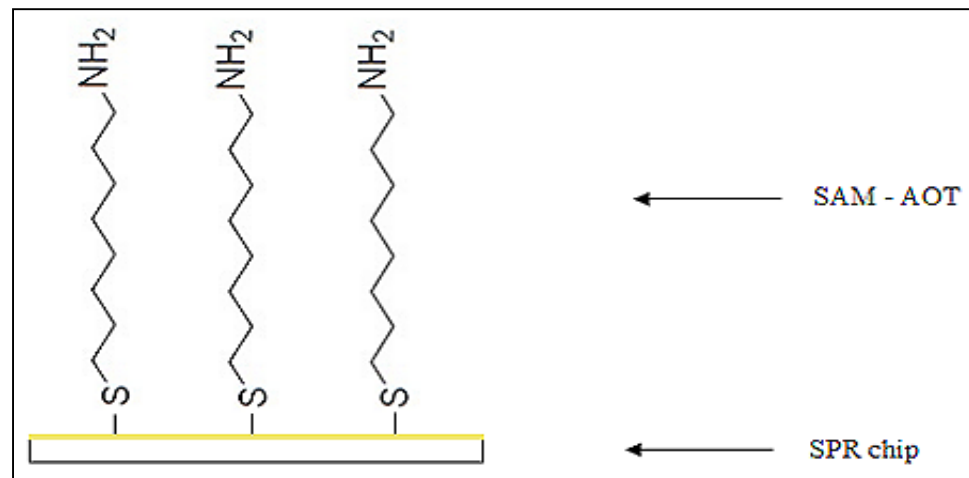


Role of Surface Plasmon Resonance (SPR) spectroscopy in molecular binding detection

Modified SPR chip:
8-amino-1-octanethiol (AOT)



Soaking in
AOT solution

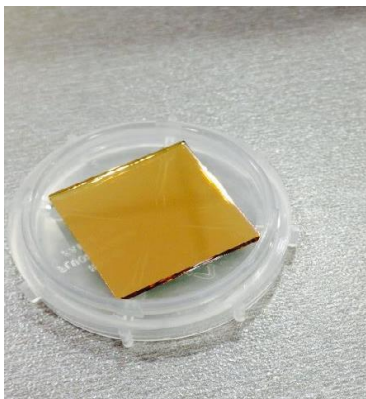


Formation of
Self-Assembled
Monolayer
(SAM) on SPR
chip

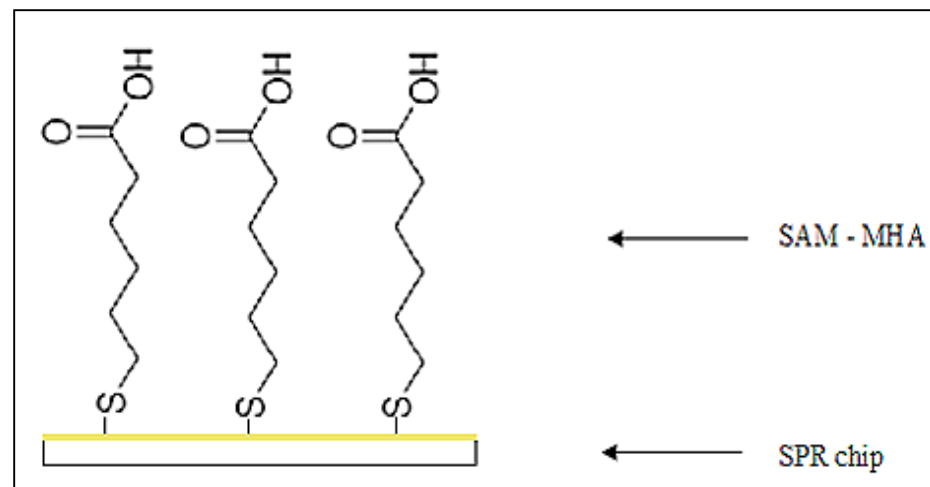
Role of Surface Plasmon Resonance (SPR) spectroscopy in molecular binding detection

Modified SPR chip:

6- mercaptohexanoic acid (MHA)

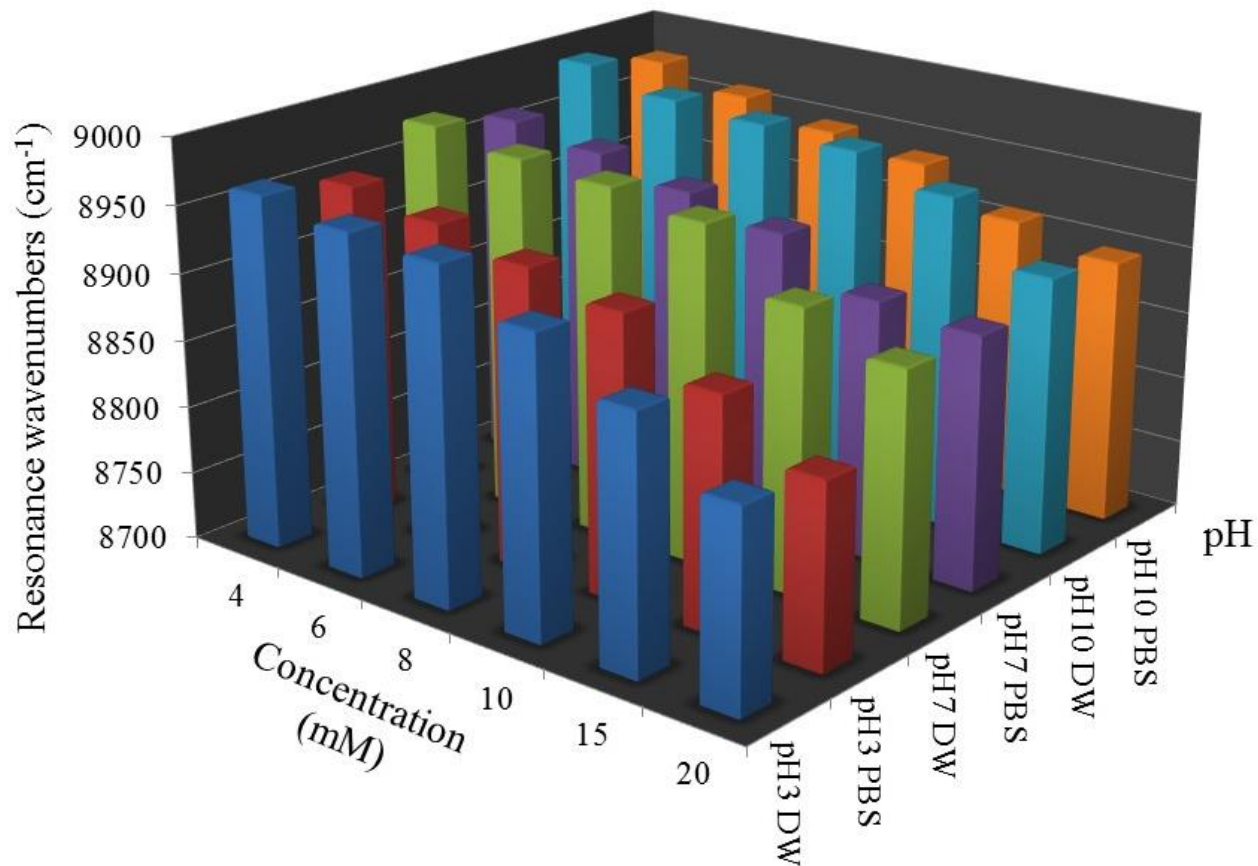


Soaking in
AOT solution



Formation of
Self-Assembled
Monolayer
(SAM) on SPR
chip

Histidine bindings on amino functionalized sensor chip

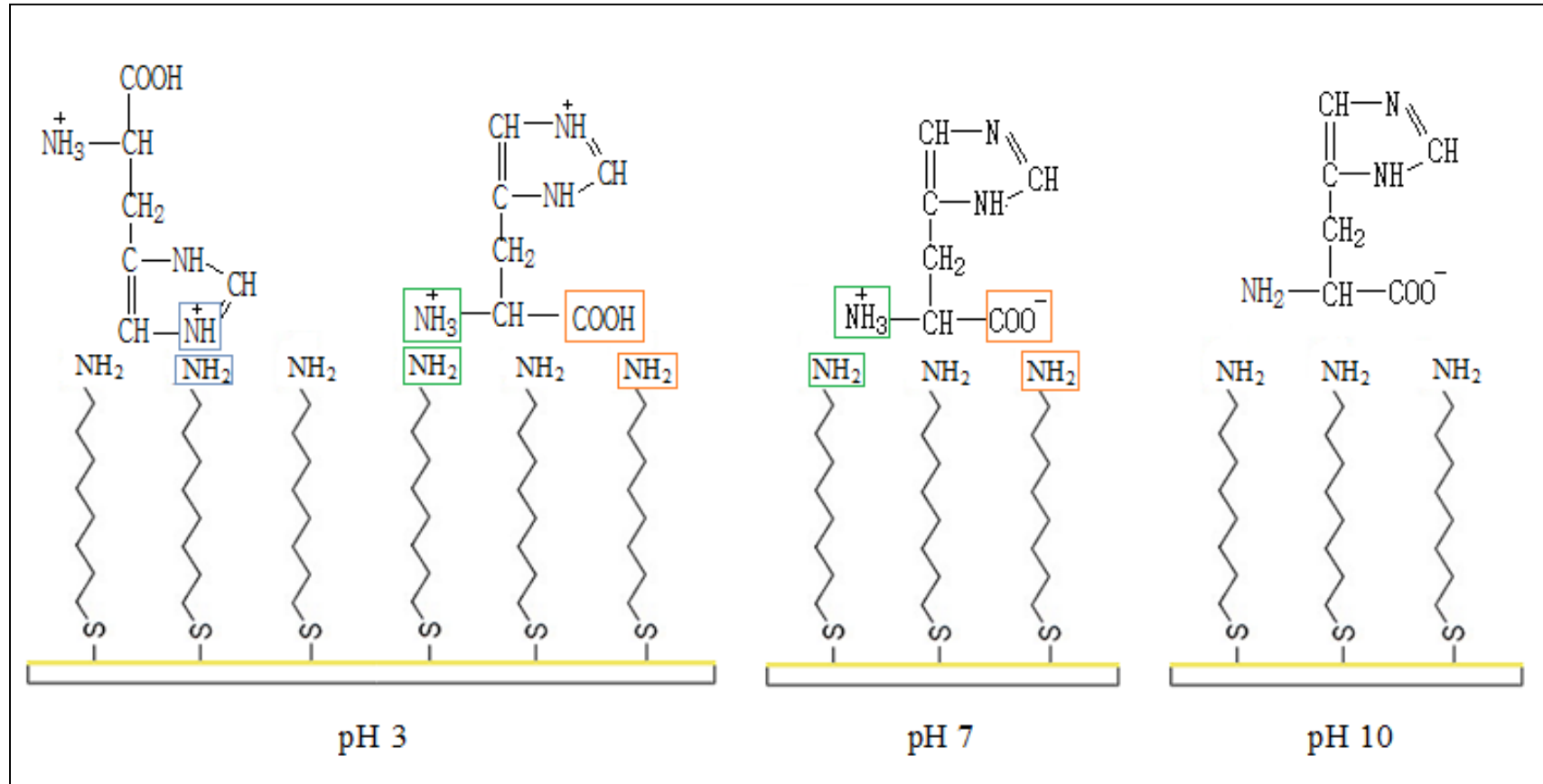


■ pH3 DW ■ pH3 PBS ■ pH7 DW ■ pH7 PBS ■ pH10 DW ■ pH10 PBS

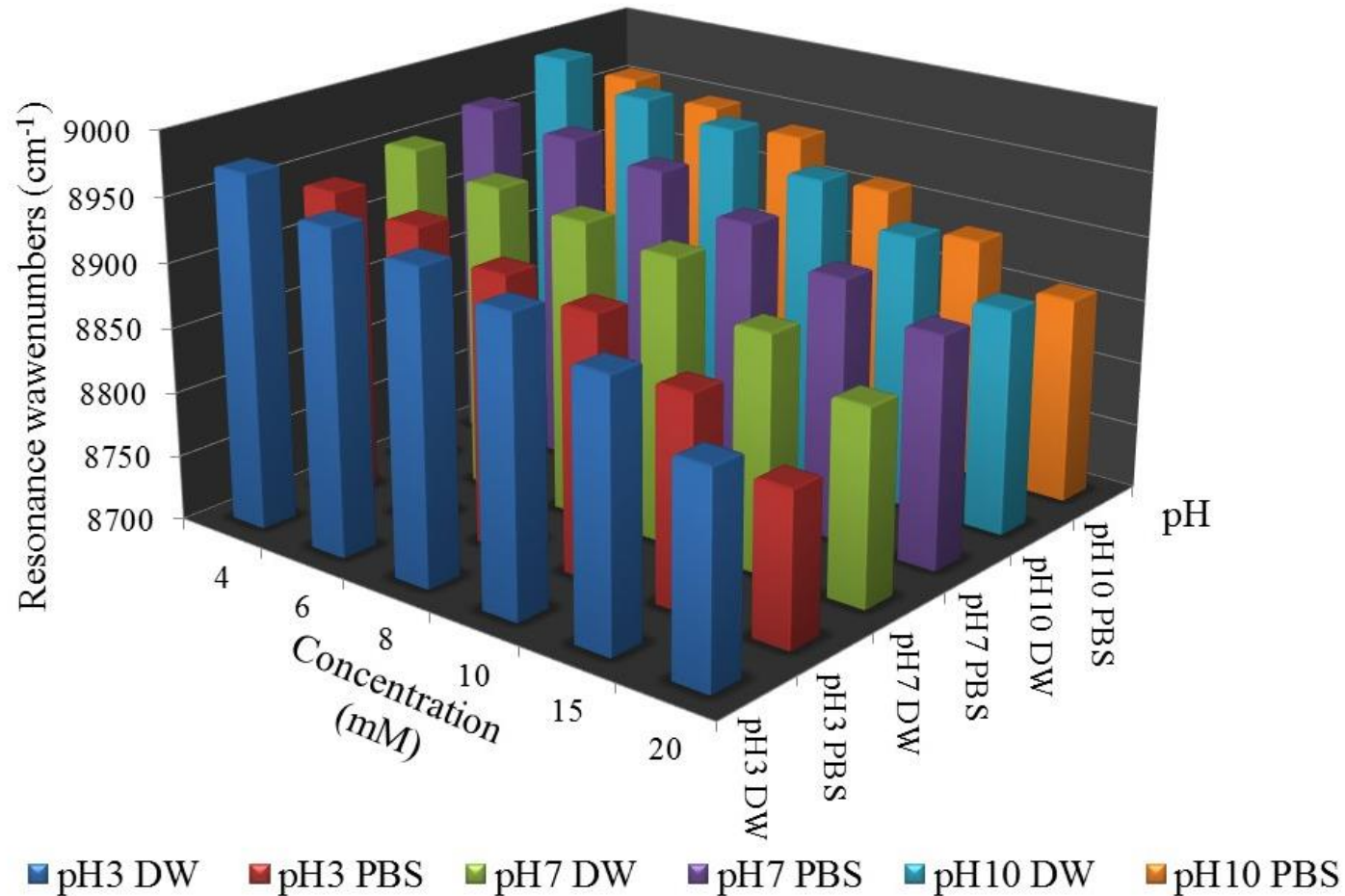
Histidine bindings on amino functionalized sensor chip surface at different concentration in distilled water (DW) and in PBS buffer at pH=3, pH=7 and pH=10



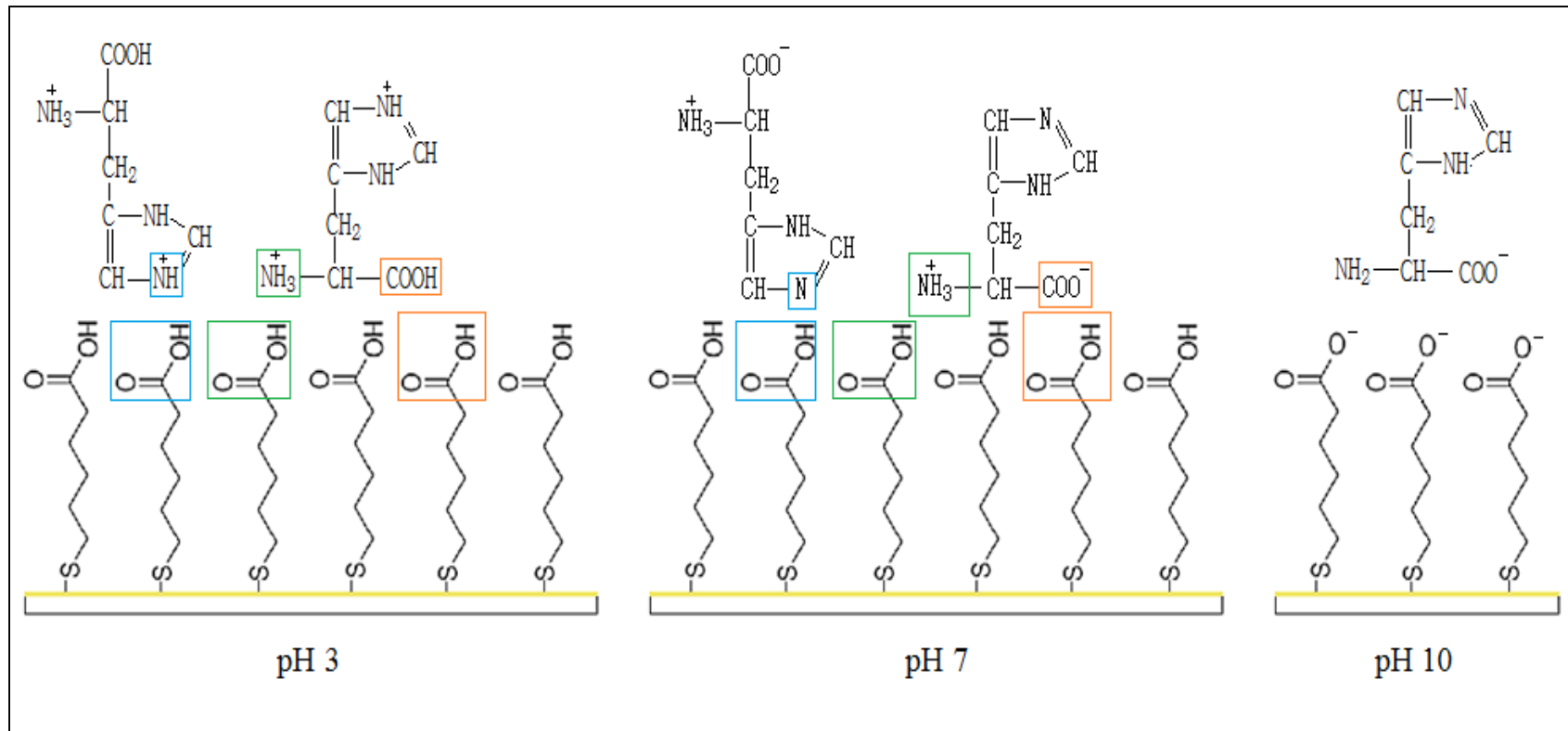
Possible interactions between AOT modified chip and histidine at different pH



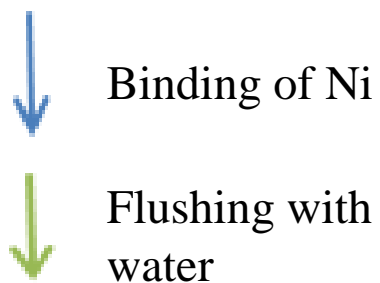
Histidine bindings on carboxyl functionalized sensor chip



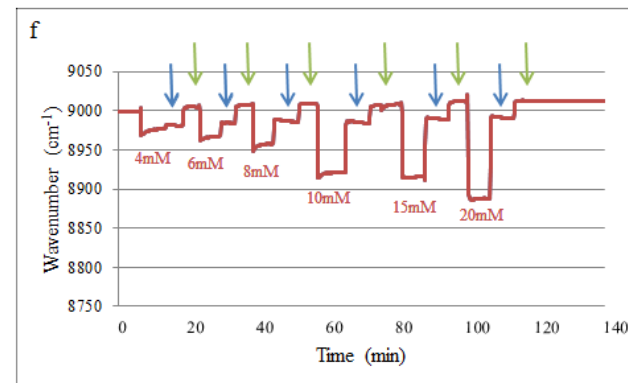
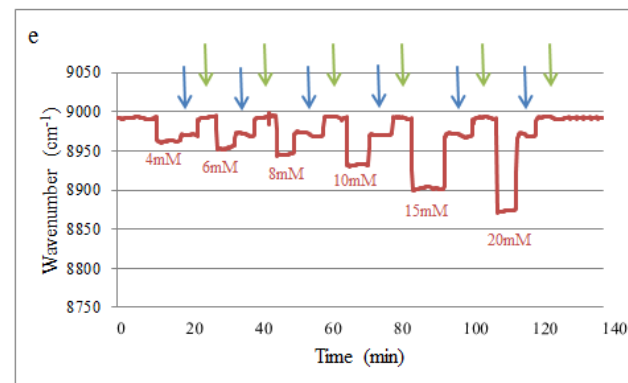
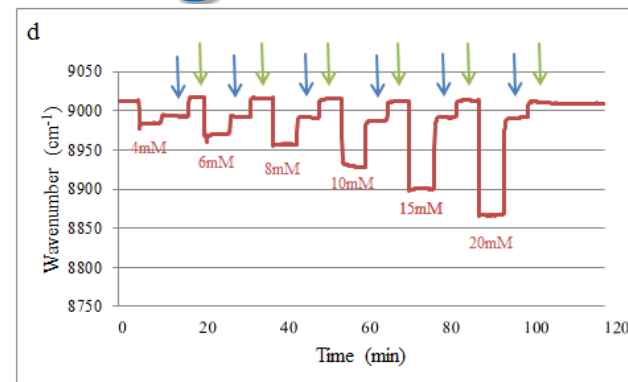
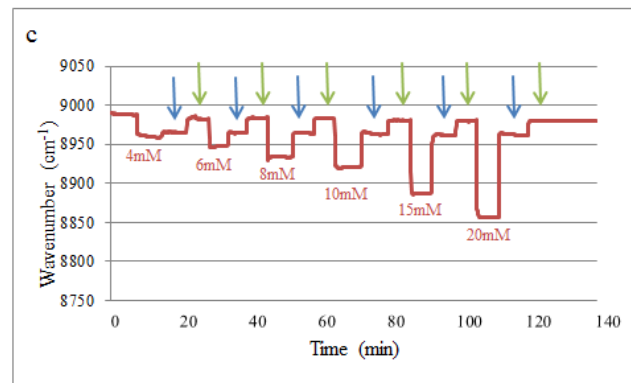
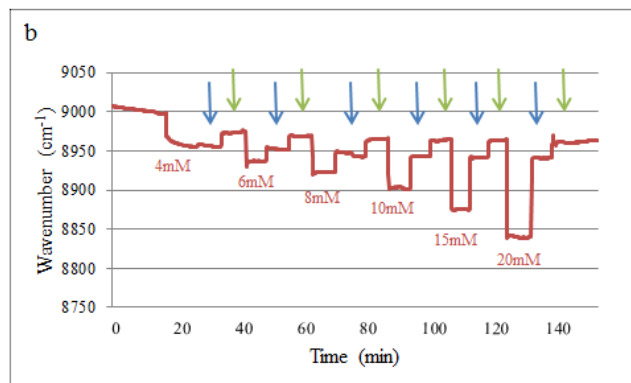
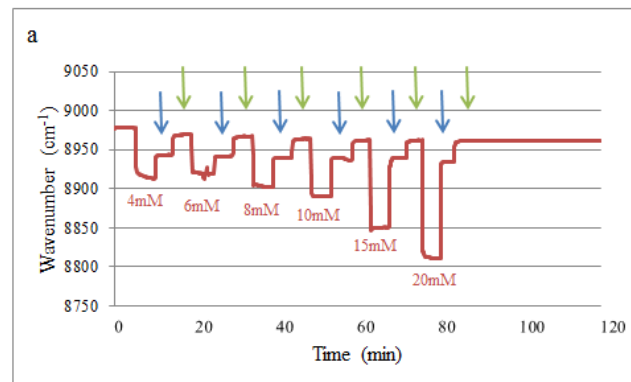
Possible interactions between MHA modified chip and histidine at different pH



Kinetic binding of Ni



Ni binding on AOT modified chip at acidic (a), neutral (b) and basic (c) pH.



Ni binding on MHA modified chip at acidic (d), neutral (e) and basic (f) pH.

Conclusions

- According to the results it has established that histidine has higher affinity to carboxyl functionalized surface in acidic conditions. Based on this study the SPR is a suitable tool to investigate the binding affinity between amino acids and dental allergens on SAM. These findings can contribute to a better understanding of dental allergen mechanism.

References

REFERENCES

1. Schmalz G. et al., *Biocompatibility of Dental Materials*, Springer, 2009
2. Kurowska E. et al., *J. Inorg. Biochem.* 105:1220-1225, 2011
3. Gree RJ. et al., *Biomater.* 21:1823-1835, 2000
4. Love JC. et al., *Chem. Rev.* 105:1103-1169, 2005

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