OSLOBAĐANJE POTENCIJALNO TOKSIČNIH KOMPONENTI IZ AKRILATA ZA MEKO PODLAGANJE PROTEZA

A RELEASE OF POTENTIALLY TOXIC COMPONENTS FROM THE ACRYLIC RESINS FOR SOFT RELINING DENTURES

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Sažetak

Uvod: Meki akrilati pripadaju grupi hladno polimerizovanih materijala koji se aplikuju na bazalnu površinu zubne proteze kako bi se eliminisale mehaničke iritacije, omogućilo ozdravljenje oštećene ili inflamirane sluzokože ili se oralno tkivo pripremilo (kondicioniralo) za prihvaćanje nove nadoknade.

Cilj istraživanja bio je ispitivanje ritma oslobađanja komponenti mekih akrilata u tri različita modela veštačke pljuvačke, u toku tridesetodnevnog opservacionog perioda.

Materijal i metode: U ispitivanju su korišćena dva meka akrilata: poli (etil metakrilat)/ n-butil metakrilat i poli (etil metakrilat)/ metil metakrilat, koji su odlagani u tri različita modela veštačke pljuvačke, u okviru tri opservaciona perioda: jedan, sedam i trideset dana. Ispitivanje je obuhvatio detekciju metil metakrilata, etil metakrilata, butil metakrilata, di butil flata i benzol peroksida tečnom hromatografijom pod visokim pritiskom.

Rezultati: Količina oslobađenih komponenti srazmerno se povećava sa porastom trajanja opservacionog perioda, bez obzira na model veštačke pljuvačke. Najviše vrednosti svih ispitivanih parametara uočene su nakon tridesetodnevnog opservacionog perioda. Model veštačke pljuvačke nije uticao na ritam oslobađanja komponenti, što znači da se one nesmetano oslobađaju u usnu duplu bez obzira na sastav pljuvačke pacijenta.

Zaključak: Sa porastom duline opservacionog perioda došlo je i do očvršavanja materijala, čime se završava njihova upotrebna vrednost.

Ključne reči: meksiakrilati, potencijalnotoksične supstance, HPLC

Abstract

Introduction: Soft acrylic resins belong to a group of cold curing materials that are applied to the basal surface of the dental prosthesis in order to eliminate mechanical irritation, and to allow for the recovery of damaged or inflamed mucous membranes, and prepare the oral tissue to accept a new compensation.

The aim of the study was to examine the rhythm of the release of soft acrylic resin components in three different models of artificial saliva, during a thirty-day observation period.

Material and Methods. Two soft acrylic resins were used in the study: poly (ethyl methacrylate)/n-butyl methacrylate and poly (ethyl methacrylate)/methyl methacrylate, which were deposited in three different models of artificial saliva within the three observation periods: one, seven and thirty days. The test involved the detection of methyl methacrylate, ethyl methacrylate, butyl methacrylate, dibutyl phthalate and benzyl peroxide under high-pressure liquid chromatography.

Results: The amount of released components increased proportionally with the increase in the duration of the observation period, regardless of the artificial saliva model. The highest values of all tested parameters were detected after a thirty-day observation period. The artificial saliva model did not affect the release of the components, which means they are freely released into the oral cavity regardless of the saliva composition of the patient.

Conclusion: As the length of the observation period increased, the material was solidified, thus ending their use value.

Key words: soft acrylic resins, potentially toxic substances, HPLC

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**Uvod**

Meki akrilati pripadaju grupi hladno polimerizovanih materijala koji se aplikuju na bazalnu površinu zubne proteze kako bi se eliminisale mehaničke irritacije, omogućilo ozdрављење оšтећене или inflamirane sluzokože ili se oralno tkivo pripremilo (kondicioniralo) za prihvatavanje nove надокнаде. Meki akrilati ostaju rezilijentni određeni vremenski period i zahvaljujući svojim viskoelastičnim svojstvima obezbeđuju podjednaku raspodelu masti-katornih sila na potporna tkiva, te na taj način uklanjaju bol i tegobe kod nosioca proteza sa veoma redukovanim, oštrim i podminiranim alveolarnim grebenima i preosetljivom или pokretnom sluzokožом1,2.

Osnovni polimer (prah) dvokomponentnog sistema mekih akrilata najčešće je poli(etil-metakrilat) (PEMA) или poli (metil-metakrilat) (PMMA). Tečnost predstavlja mešavinu estarskih plastifikatora (dibutilftalat (dBuFt), butil-glikolat) koji čine 30-60% и etanola, kao rastvarača, čiji sadžaj iznosi 4-60% 2,3. Plastifikatori, koji se dodaju akrilatnim smolama, čine ove materijale mekim na temperaturi tela4. Plastifikatori su rastvorljivi u oralnim tečnostima, a njihov postepeni gubitak vremenom vodi ka otvrdnjavanju materijala u ustima pacijenta5. Aktivator hladne polimerizacije je benzoil peroksid (BP).

Pri mešanju praha и tečnosti čestice polimera apsorbuju etanol. Čestice PEMA manje molekulske mase omogućavaju bržu и obimniju penetraciju alkohola unutar polimera u odnosu на klasični PMMA. Sa druge strane, dejstvom međumolekulskih Van der Valsovih sila polimera se separiraju, pa se veliki molekuli plastifikatora interpoliraju između njih. Nakon homogenizacije cela masa dobija želatinoznu formu leća. Plastifikatori u rastvorljeni јавном, a njihov postepeni gubitak vremenom vodi ka otvrdnjavanju materijala u ustima pacijenta. Aktivator hladne polimerizacije je benzoil peroksid (BP).

Oslabljavanje etanola из полимеризованог kondicionera у oralnu средину започинje непосредно након предаје протезе пациенту, што се компенсује асорпциjom vode2,3. Kako se gubitak etanola и асорпциja vode не одгравају истовремено и истом brzinom, физичка svojstva materijala se vremenom menjaju. Nakon окончавања асорпционог postupka, materijal potpuno očvrse, па ga je potrebno zameniti или protezu koristiti као definitivnu 4,5.

**Introduction**

Soft acrylic resins belong to a group of cold curing materials that are applied to the basal surface of the dental prosthesis in order to eliminate mechanical irritation, and to allow for the recovery of damaged or inflamed mucous membranes, and prepare the oral tissue to accept a new compensation. Soft acrylic resins remain resistant for a certain period of time and, thanks to their viscoelastic properties, provide an equal distribution of the masticatory forces to the supporting tissues, thus removing the pain and distress of the prosthesis carrier with very reduced, sharp and submerged alveolar ridge and hypersensitive или movable mucous membrane1,2.

The basic polymer (powder) of a two-component soft acrylate system is usually poly (ethyl methacrylate) (PEMA) или poly (methyl methacrylate) (PMMA). The liquid is a mixture of ester plasticizers (dibutyl-phthalate (DBP), butyl glycolate) consisting of 30-60% of ethanol as a solvent, which content is about 4-60%2,3. Plasticizers, which are added to acrylic resins, make these materials soft at body temperature 4. Plasticizers are soluble in oral fluids, and their gradual loss in time leads to the hardening of the material in the mouth of the patient5. The cold curing agent is benzoyl peroxide (BP).

Ethanol is absorbed by the polymer particles when mixing the powder with the liquid. The particles of the PEMA with smaller molecular weight allow for faster и more extensive penetration of alcohol within the polymer than the conventional PMMA. On the other hand, by the action of intermolecular Van der Waals forces, the chains of the polymer are separated, so that the large molecules of the plasticizer interpenetrate between them. After homogenization, the whole mass is obtained by gelatinous form due to the chains of polymers trapped inside the matrix composed of ethanol и plasticizers 1.

The release of ethanol из полимеризованог conditioner into the oral environment begins immediately after giving the dentures to the patient, which is compensated by water absorption2,3. Since the loss of ethanol и water absorption do not occur simultaneously and at the same speed, the physical properties of the material change over time. After the end of the absorption process, the material is fully hardened, so it should be replaced или the dentures used as a definitive one6,7.
Komponente koje ulaze u sastav mekih akrilata u literaturi mogu biti toksične za oralna tkiva ukoliko se u potpunosti ne neutralizuju u procesu polimerizacije8-11. Kako ne postoji apsolutno vezivanje materijala u toku njegove pripreme i izrade zubnih proteza, u istraživanju se krenulo od pretpostavke da će u vremenu korišćenja mekih akrilata kao lajnera doći i do oslobađanja komponenti u okolni tečni medijum.

Cilj istraživanja bio je ispitivanje ritma oslobađanja komponenti mekih akrilata u tri različita modela veštačke pljuvačke, u toku tridesetodnevnog opservacionog perioda.

### Materijal i metode

U ispitivanju su korišćena dva meka akrilata: **Lang Flexacryl** (PEMA/ n-butyl metakrilat) i **Lang Immediate** (PEMA/ metil metakrilat) (Lang Dental MFG.Co., SAD). Od oba materijala napravljeno je po 27 uzoraka dimenzija 10 x 10 x 2 mm prema uputstvu proizvođača, u kalupu od kondenzacionog silikona. Nakon izrade uzorci su pokazivali očekivanu rezilijentnost.

Uzorci su podeljeni u tri eksperimentalne grupe od po 3 uzoraka, koji su potapani u tri različita modela veštačke pljuvačke (tabela 1), u toku tri opservacionih perioda: jedan, sedam i trideset dana ($n=27$).

According to the literature, the components included in the structure of soft acrylic resins may be toxic to oral tissues if they are not completely neutralized in the polymerization process8-11. Since there is no absolute bonding of material during its preparation and development of dental prostheses, the research started from the assumption that at the time of using soft acrylates as a liner, the components will be released into the surrounding liquid medium.

**The aim** of the study was to examine the rhythm of the release of soft acrylate components in three different models of artificial saliva, during a thirty-day observation period.

### Material and methods

Two soft acrylic resins that were used during the study are **Lang Flexacryl** (PEMA/n-butyl methacrylate) and **Lang Immediate** (PEMA/methyl methacrylate) (Lang Dental MFG Co., USA).

27 samples were made of both materials (dimension: $10\times10\times2$ mm) according to the manufacturer's instructions, in a condensation silicone mold. After making the samples, they showed the expected resilience.

Samples were divided into three experimental groups consisting of three samples, submerged in three different models of artificial saliva (Table 1), during three observation periods: one, seven and thirty days ($n=27$).

| Table 1. Models of artificial saliva |
|-------------------------------------|
| **Model 1**                         | **Model 2**                         | **Model 3**                         |
| g komponenti / l deionized water    | g components / l deionized water    | natrijum hordan karmonat            |
| ksanthan gum                        | 0.18                                | potassium chloride                 |
| kalijum hlorid potassium chloride   | 1.20                                | 0.18                                |
| natrijum hlorid sodium chloride     | 0.85                                | natrijum hordan potassium chloride  |
| magnezijum hlorid magnesium chloride| 0.05                                | 0.85                                |
| kalijum hlorid calcium chloride     | 0.13                                | kalijum karbonat potassium carbonate|
| di kalijum hidrogen ortofosfat      | 0.13                                | 0.12                                |
| metil p-hidroksibenzoat methyl p-hydroxybenzoate | 0.35 | 0.35 |
| α amilaza                           | 0.20                                | 0.20                                |
Model 1 i 2 međusobno se razlikuju u sadržaju enzima, α amilaze. Inkubacija uzoraka u veštačkoj pljuvačci obavljena je u zatvorenim plastičnim posudama, u vodenom kupatilu, na temperaturi 37±10°C. Odnos mase uzoraka i zapreminе veštačke pljuvačke iznosio je 0,1 g materijala / 1ml veštačke pljuvačе (ISO 10993-5: 199914-16).

Količina oslobođenih supstanci ispitivana je u modelu pljuvačке nakon uklanjanja uzorka posle svakog od navedenih perioda. Ispitivanje je obuhvatilo detekciju metil metakrilata (MMA), etil metakrilata (EMA), butil metakrilata (BuMA), di butil ftalata (dBuFt) i benzoil peroksida (BP).

Korišćeni uređaj za tečnu hromatografiju pod visokim pritiskom (HPLC) je Agilent 1100 Series (USA), sa DAD 1200 detektorom i analitičkom kolonom SUPELCO Discovery HS C18 250 × 4,6 mm, 5 µm, Sigma -Aldrich, SAD. Kao eluent poslužio je metanol. Protok mobilne faze iznosio je 1 cm3/min, a zapremina injektiranja uzorka 20 µl. Kolona je termostatirana na 25°C. Talasna dužina detekcije bila je 205 nm.

Uzorci rastvora tri modelа veštačke pljuvačke korišćeni su za HPLC analizu bez prethodne posebne obrade. Uzorci su filtrirani na ekono-filteru prečnika pora 0,45 µm, nakon čega je po 20 µl injektirano u HPLC uređaj.

Kalibracionе krive su izrađivane od serije rastvora svakе od ispitivaniх supstanci u metanolу. Početna koncentracija ispitivanog jedinjenja bila je oko 1 mg/cm3, od koje je, zatim, razblaživanjem metanolom pravljena serija rastvora manjih koncentracija. Iz dobijenih hromatogramа očitavani su potrebni podaci: retencionо vreme svakог jedinjenja (Rt) и površina pika (A). Uređaj očitava UV/VIS spektar u svakoj tački pika na hromatogramu, tako da se iz spektара može odreditи i vrednost λmax, tj. talasna dužina na kojoj jedinjenje ima maksimalnu apsorbancu.

Koncentracija ispitivanih komponenti u modelima vešтаčке pljuваčке предстаљена као µg supstance/cm3 rastvora плjuвачке. Koncentracije су сагледаване кроз средњу vrednost sa standardnim devijacijama. Vršена je komparacija dobijenih vrednosti koncentracija unutar istе grupe materijala u zavisnosti od dužine inkubacionog perioda, kao и између два ispitivana materijala.

Model 1 and 2 differ in the content of the enzyme, α amylase. Incubation of samples in artificial saliva was performed in closed plastic containers, in a water bath, at a temperature of 37 ± 10°C. The mass ratio of samples and volume of artificial saliva was 0.1 g of material/1 ml artificial saliva (ISO 10993-5:1999)14-16.

A quantity of released substances was examined in the saliva model after removal of the sample after each of the mentioned periods. The study involved the detection of methyl methacrylate (MMA), ethyl methacrylate (EMA), butyl methacrylate (BMA), dibutyl phthalate (dBuFt) and benzoyl peroxide (BP).

Agilent 1100 Series (USA), with the DAD 1200 detector and the analytical column SUPELCO Discovery HS C18 250×4.6 μm, 5 mm, Sigma-Aldrich, USA was used as a high-pressure liquid chromatography device (HPLC). The methanol was used as an eluent. The flow rate of the mobile phase was 1 cm3/min, and the sample injection volume was 20 ml. The column is thermostated at 25°C. The wavelength of detection was 205 nm.

The samples of the solution of three models of artificial saliva were used for HPLC analysis without prior processing. Then, the samples were filtered on a 0.45 mm pore diameter filter, which was followed by an injection of 20 µl into an HPLC device.

The calibration curves were made from a series of solutions of each of the tested substances in methanol. The initial concentration of the tested compound was about 1 mg/cm3, from which, after dilution with the methanol, a series of solutions of lower concentrations were made. The required data from the obtained chromatograms were read out: the retention time of each compound (Rt) and the surface of the peak (A). The device reads the UV / VIS spectrum at each peak point on the chromatogram, so that the λmax value can be determined from the spectrum i.e., the wavelength at which the compound has a maximum absorbance.

The concentration of the tested components in artificial saliva models is presented as µg of the substance/cm3 of the saliva solution. Concentrations were viewed through a mean value with standard deviations. A comparison was made of the obtained concentration values within the same group of materials, depending on the length of the incubation period, as well as between the two tested materials.
**Rezultati**

U Tabelama 2 i 3 prikazane su dobijene vrednosti oslobodnih komponenti ispitivanih materijala u tri modela veštačke pljuvačke HPLC metodom, nakon tri opservaciona perioda.

Količina oslobodnih komponenti srazmerno se povećava sa porastom trajanja opservacionog perioda, bez obzira na model veštačke pljuvačke. Veća koncentracija oslobodnih komponenti uočena je kod materijala Lang Immediate.

U tumačenju rezultata treba naglasiti i činjenicu da su uzorci sa porastom dužine opservacionog perioda menjali svoju konzistenciju, te su nakon tridesetdana postali čvrsti, izgubivši rezilijentnost.

**Results**

Table 2 and table 3 show the obtained values of the released components of the tested materials in three models of artificial saliva using HPLC method after three observation periods.

The amount of released components increases proportionally with the increase in the duration of the observation period, regardless of the artificial saliva model. A higher concentration of released components was detected in Lang Immediate material.

In interpreting the results, it should be emphasized that the samples with the increase in the length of the observation period changed their consistency, and after thirty days they became firm, losing their resilience.

**Tabla 2.** Srednje vrednosti i standardne devijacije količina oslobodnih komponenti u uzorcima različitih modela veštačke pljuvačke nakon ekstrakcije uzoraka Lang Flexacryl

**Table 2.** Mean values and standard deviations of the quantities of released components in samples of various models of artificial saliva after the extraction of Lang Flexacryl samples
**Discussion**

This study involved the analysis of three different artificial saliva solutions in terms of detection of the required components by HPLC method.

In proportion to the time of disposal, there has been an increase in the concentration of released components in saliva models, which also indicates their decrease in the samples itself, and also the increase in their biocompatibility. The artificial saliva model did not affect the trend of the obtained results, nor the addition of the α amylase enzyme. In accordance with the obtained results, it can be concluded that the composition of the saliva does not affect the release of unbound components from the soft acrylic resins.

Previous studies have indicated the potentially toxic effect of certain unbound substances from acrylic materials. Research by Kostic et al. indicated a decrease in the amount of these components in the samples of the material after their immersion in the aqueous environment and the post-polymerization procedures.

An unbound or residual monomer, MMA or EMA may have an allergic or irreversible effect. It is difficult to predict the individual tolerant level of the residual monomer. The amount of residual MMA should range from 1 to 3%. According to the standard (ISO 1567:1999), the maximum permissible amount of residual MMA for...
BP se koristi u dermatologiji, gde postoje opisani slučajevi kontaktnog dermatita u oko 1% slučajeva\(^{23-25}\). Oyama i Imai su ukazali na citotoksični efekat BP\(^{26}\). Kompletna količina BP se ne utroši u postupku pokretanja polimerizacije akrilata, mada se on prahu akrilatnog materijala dodaje u vrlo niskim koncentracijama 0,2 i 1,28%\(^{23,25}\). BP nije pokazao značajnije varijacije u količini oslobodenoj iz uzoraka Lang Immediate i Lang Flexacryl. Uočeno je ravnomerno oslobađanje iz uzoraka akrilata, a najveća koncentracija u pljuvačci bila je nakon trideset dana inkubacije uzoraka.

Dokazana je i citotoksičnost ftalata, plastifikatora akrilatnim materijalima\(^{26-30}\). Vrednosti oslobodene količine plastifikatora (dBuFt) nakon prvog dana potapanja, u uzorcima mekih akrilata, takođe su slične. U slučaju Lang Immediate, količina dBuFt ravnomerno raste sa porastom dužine inkubacije. Dobijene vrednosti su, nakon trideset dana, manje u odnosu na Lang Flexacryl. Kod Lang Flexacryl nagli porast koncentracije plastifikatora odvija se između sedmog i tridesetog dana potapanja. Sa oslobađanjem plastifikatora u rastvor veštačke pljuvačke, evidentirane su promene fizičkih karakteristika mekih akrilatnih materijala.

U cilju poboljšanja biokompatibilnosti mekih akrilata sintetisani su i kondicioneri bez ftalata, čiji je nedostatak brz gubitak viskoelastičnosti. Sintetisan je i oralni kondicioner bez etanola, dobijen kombinacijom vinil estara i PEMA\(^2\). Nije moguće precizno odrediti standardne vrednosti minimalnih količina analiziranih komponenti akrilatnih materijala koje bi mogle izazvati toksičnu ili alergijsku reakciju oralnog tkiva. Sa druge strane, u koncentrovanoj obliku oni pokazuju jak toksični efekat. Obzirom na dokazano toksično dejstvo, treba težiti njihovom maksimalnom smanjenju strogim poštovanjem odnosa praha i tečnosti, polimerizacionog postupka propisanog od strane proizvođača i odlaganjem nadoknada od hladno polimerizovanih akrilata u vodu nekoliko dana pre predaje pacijentu\(^{31-33}\). Cytotoxicity of phthalate, a plasticizer of acrylate materials was also demonstrated\(^{26-30}\). The values of the released amount of plasticizer (dBuFt) after the first day of immersion, in soft acrylate samples, are also similar. In Lang Immediate case, the amount of dBuFt increases uniformly with the increase of the incubation period. The values obtained after thirty days were less compared to the Lang Flexacryl model. In Lang Flexacryl case, a sudden increase in the concentration of the plasticizer occurs between the seventh and thirtieth day of immersion. With the release of the plasticizer into artificial saliva, changes in the physical characteristics of soft acrylic materials were recorded.

In order to improve the biocompatibility of soft acrylic resins, the conditioners without phthalate were synthesized, whose deficiency is a rapid loss of viscoelasticity. Oral conditioning without ethanol is also synthesized, obtained by the combination of vinyl esters and PEMA\(^2\).

It is not possible to accurately determine the standard values of the minimum quantities of analyzed components of acrylic materials that could cause a toxic or allergic reaction of the oral tissue. On the other hand, in a concentrated form, they show a strong toxic effect.

Given the proven toxic effect, the maximum reduction by strict observance of the powder and liquid ratio is very important. It is done through the polymerization procedure prescribed by the manufacturer and the disposal of prosthetics made of cold curing acrylics in water for several days before handing over to the patient\(^{31-33}\).
Zaključak

Ispitivane potencijalno toksične supstance ravnomerno su se oslobađale u vodenu sredinu, srazmerno sa dužinom inkubacije. Najviše vrednosti svih ispitivanih parametara uočene su nakon trideseto dnevnog opservacionog perioda. Model veštačke pljuvačke nije uticao na ritam oslobađanja komponenti, što znači da se one nesmetano oslobađaju u usnu duplju bez obzira na sastav pljuvačke pacijenta. Veća koncentracija oslobodjenih komponenti uočena je kod materijala Lang Immediate.

Sa porastom dužine opservacionog perioda došlo je i do očvršavanja materijala, čije se završava njihova upotrebna vrednost. Inflamatorni efekat komponenti mekih akrilata na oralnu sluzokožu biće predmet budućih istraživanja.

Conclusion

Tested potentially toxic substances were uniformly released into the aquatic environment, in proportion to the incubation length. The highest values of all tested parameters were detected after a thirty-day observation period. The artificial saliva model did not affect the release of the components, which means they are freely released into the oral cavity regardless of the composition of the patient's saliva. A higher concentration of released components was detected in the Lang Immediate material.

As the length of the observation period increased, the material was solidified, thus ending their use value.

The inflammatory effect of the soft acrylic components on the oral mucosa will be the subject of future research.

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