Research Design of the Release of Amizon, Decametoxine, and Chlorhexidine from the Composition of Dental Medicinal Films

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Примітка [d1]: Orcids?

ARTICLE INFO

ABSTRACT

Introduction: The treatment of most diseases in dentistry is related to the local application of antiseptic drugs that allow regulating the microbiocenosis in the oral cavity. The study of pharmacodynamic and kinetic parameters of the release of active pharmaceutical ingredients (API) from the dosage form (DF) is an important task. In this study, we tried to evaluate the release of API from developed dental medicinal films (DMF) produced on a polymeric basis.

Methods: To confirm the antimicrobial activity of the three developed DMF with amizon, decamethoxine and chlorhexidine digluconate, a statistical analysis and mathematical processing of the research results were carried out.

Results: Statistical processing made it possible to organize the results of our earlier studies, in which the concentrations of three API (amizon, decamethoxine and chlorhexidine digluconate) were determined during their diffusion from the polymer base of the DMF to the aqueous dispersion medium (0.9% sodium chloride solution). Due to performed statistical calculations, regarding the reliability of API release, it was determined that the time duration of complete release of the three active substances from the polymer base were different, which will ultimately affect the duration of the pharmacological effect of the API in the patient's body.

Conclusion: In the manufacture of DMF on a polymeric base of hydrophilic type, it is necessary to take into account the release time of each API, according to the composition of the polymeric coatings.

Intorduction

Oral and dental ailments, including tooth decay, dental caries, gingivitis, periodontitis, commonly occur on the globe and are often caused by bacterial infections. Different antiseptics of synthetic and natural origin as well as other groups of medicines are applied in periodontal diseases and tooth infections, due to their inhibitory effect on the growth of *Escherichia coli, Staphylococcus aureus, Streptococcus mutans, Streptococcus sobrinus, Lactobacillus spp., Candida ssp.* and other microorganisms, anti-inflammatory, analgesic and wound-healing activities, ability to prevent dental plague formation (Wang et al., 2014;

Gasmi Benahmed et al., 2020; Manouchehri, 2022; Gasmi Benahmed et al., 2022a; Santacroce et al., 2022; Mandil et al., 2023; Manouchehri et al., 2023; Mandil et al., 2023). Antimicrobial properties of applied dental biomaterials can decrease pathogenic contamination and local inflammatory processes (Gasmi Benahmed et al., 2022b).

Anatomical and physiological features of the oral cavity complicate the use of most DF, since the oral cavity is lined with smooth epithelial tissue (mucous membrane), which is constantly washed with oral fluid (saliva). As a result, the local application of any medicinal products (solutions, ointments, gels or pastes) is accompanied by the washing out from the mucous surface and impossibility of long-term fixation, as well as a decrease in the concentration of the active substance (in the composition of the medicinal product) due to its dilution with oral fluid (Kida et al., 2021).

Therapeutic assistance to dental patients is an important component when considering human health as a whole (Lamster and Eaves, 2011; Sanders et al., 2021; Katakura, 2022). It is possible to optimize and facilitate the therapy of dental patients due to the development of a new DF in the form of DMF, which after application to the mucous membrane undergo a gradual bio-destruction and release of API (Rokaya et al., 2018; Petrescu et al., 2022; Zhang et al., 2022).

The reliability of the release of API from the DMF in the test solution, allows mathematical and statistical evaluation of the experiment *in vitro*, which can predict the optimal time of their dissolution in the human mouth under physiological norms and to simulate the results of research close to *in vivo* conditions (Vasylenko and Sencha, 2011).

Analysis of the results for statistical evaluation of the release of amizon, decametoxine and chlorhexidine will allow the production of DF applied in the form of DMF, the advantage of which comprise a controlled period for the API release and their diffusion through the oral mucosa without compromising the integrity of epithelial tissues.

Therefore, the aim of this work was to perform statistical processing, using previously obtained results of sampling studies, and to determine the concentrations of three API that diffused to the dispersion medium (aqueous sodium chloride 0.9%) from the produced DMF with amizon, decametoxine and chlorhexidine digluconate.

Materials and Methods

Comparison of the release from the composition of DMF containing amizon, decametoxine and chlorhexidine digluconate, depending on the time in the dynamics was performed by mathematical confirmation of the results obtained from three studied groups of DMF, with different active substances - 6 test samples in each group, totally eighteen polymer films were prepared on a polymeric hydrophilic basis.

Statistical data processing, based on the Tukey method, was used to study and compare the time periods of API release, from the composition of DMF, yielding amizon, decametoxine and chlorhexidine digluconate, since these indicators can affect their pharmacokinetics and pharmacodynamics.

Results:

Statistical evaluation of the rate of API release was performed by studying three DMF of the following compositions (Table 1).

Application of mathematical, statistical processing was used to confirm the preliminary results of the study on DMF sampling, in which the concentrations of amizon, decametoxine and chlorhexidine digluconate, diffused to the dispersion medium (aqueous sodium chloride 0.9% solution), were determined.

Comparison of the time parameters of release from the composition of DMF containing amizon, decametoxine or chlorhexidine digluconate in the dynamics was performed by statistical comparison of eighteen test samples in the form of polymeric films made on a polymeric hydrophilic basis (Hrynovets, 2013). Thus, sampling was carried out in time periods through 3, 5, 10, 15, 20, and 25 min (Table 1).

The average values of the ingredients at each fixed time after the release of API into the composition of an aqueous Na Cl (0.9% solution) were also determined (Figure 1). Estimation of residual variances was performed by formulating a hypothesis and further comparison of received experimental and tabulated F-values of Fischer-Snedekor distribution for reliability P < 0.05 (Horoneskul, 2009).

During the experiment from each DMF, where the content of test samples of API is expressed as a percentage, depending on the time, sampling was performed after application to the base of the polymer carrier by analysis of variance using the method of Tukey (Vasylenko and Sencha, 2011), the optimal time of dissolution of the polymer base and the transition of the gel form into a solution form were estimated. The result of the research is a statistical analysis of the ratio of time indicators, in accordance with the concentration of the active substance in the composition of the DMF in the test solution, are represented (Tables 2, 3, and 4). The table 2 summarizes the results of the release of three investigated API from DMF into the tested solution.

To obtain a statistical conclusion based on analysis of variance, the API amizon is taken here as an example.

Formulation of hypotheses and set the level of significance:

$$H_0: \mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6,$$

there is no significant difference between the average values of amizon release over time;

$H_1:$ the difference between the average values is significant; level of significance $\alpha = 0,05$.

The criteria for testing hypotheses, using statistical processing of the obtained data, is determined as follows:

$$F = MS_A / MS_W,$$

with the number of degrees of freedom

$$\nu_1 = I - 1, \ \nu_2 = n - I,$$

where the time factor is variable ($\nu_1 = 5; \ \nu_2 = 35$).

According to the algorithm, calculations on the results

of statistical analysis were performed (Table 3). Calculation of the residual variance was conducted using formulae (Bakhrushyn, 2006):

$$SS_A = \sum_{i=1}^{I} n_i (\overline{Y}_{i*})^2 - n\overline{Y}^2$$

$$SS_W = Y_{**}^2 - \sum_{i=1}^{I} n(\overline{Y}_{i*})^2 \qquad MS_A = \frac{1}{v_A} SS_A$$

$$MS_W = \frac{1}{v_W} SS_W$$

The variance analysis with empirical criterion value calculation (Anistratenko and Fedorov, 1993) is shown (Table 5).
According to the Fischer-Snedekor tables, the following results will be critical:.

$$f^* = f(p = 0,95, \quad v_1 = 5, \quad v_2 = 30) = 2,65$$

$$f^* = f(p = 0,999, \quad v_1 = 5, \quad v_2 = 30) = 4,182$$

Since $f_e > f^*$, the difference between the average values is significant, i.e. amizon release is accurate at the time (p<0.001).

To assess amizon release over time, the method of Tukey (Vasylenko and Sencha, 2011) was used, as the number of values in the groups are the same.
Formulation of hypotheses:

$$H_0: \mu_k - \mu_l = 0, \quad (k, l) = 1, 2, 3, 4, \quad k \neq l ;$$

H_1: there are non-zero slope; level of significance

The criterion of statistical hypothesis testing (Herasymenko et al., 2000) is:

$$Q_{kl} = \frac{\overline{Y}_{k*} - \overline{Y}_{l*}}{\sqrt{MS_W / m}}$$

The numbers of degrees of freedom:

$$v_1 = 5, \quad v_2 = 30$$

$$q^* = q(p = 1 - \alpha_1; \quad v_1 = I; \quad v_2 = I(m-1)).$$

$$q^* = q(p = 0,95; \quad v_1 = 6; \quad v_2 = 30) = 4,30$$

The half-width of the probability range is:

$$q^* \sqrt{MS_W / m} = 5,59$$

The received data comparison is given in Table 4.
Analysis of variance showed that the content of amizon, decametoxine, and chlorhexidine digluconate in the tested solution for each of the films significantly increased (p < 0.001).
Most quantity of amizon was released during the first 10 minutes (p < 0.001), by 15 minutes the reliability of the release decreased (p < 0.05), and subsequent its release was insignificant and included in the parameters of insignificant maintenance of concentration for 30 min from the beginning of the experiment.
Dexametoxinum is released faster during the first 10 minutes (p < 0.001), after the release is insignificant and its subsequent release was insignificant and included in the parameters of insignificant maintenance of the concentration for 30 min from the beginning of the experiment.
Chlorhexidine digluconate is released to the greatest degree in the first 15 minutes (p < 0.001), up to 20 minutes the reliability decreases (p < 0.05), and after 20 minutes it becomes not statistically significant (p > 0.05), insignificant and included in the parameters of insignificant maintenance of concentration for 40 minutes from the beginning of the experiment.

Table 1. Release of active pharmaceutical ingredients from the composition of the dental medicinal films into the experimental aqueous solution (NaCl 0.9%).

Time sampling (min)	The content of API in the experimental samples,% of the content in the DMF		
	Amizon	Decamethoxinum	Chlorhexidine digluconate
3	49.7 ± 0.01	70.0 ± 0.1	7.0 ± 0.01
5	71.4 ± 0.01	85.0 ± 0.05	17.0 ± 0.03
10	86.8 ± 0.01	90.0 ± 0.1	28.0 ± 0.02
15	90.6 ± 0.01	95.0 ± 0.05	49.0 ± 0.01
20	92.7 ± 0.01	96.7 ± 0.1	75.0 ± 0.02
25	93.0 ± 0.01	97.0 ± 0.1	80.0 ± 0.01

API: Active pharmaceutical ingredients; DMF: Dental medicinal films; Solution was NaCl 0.9%.

Table 2. Release of amizon, decametoxine and chlorhexidine digluconate from dental medicinal films into the tested solution

Time sampling after preparation of DMF, min	The average value of the content of the API in experimental samples of different DMF		
	Amizon	**Decamethoxine**	**Chlorhexidine digluconate**
3	49.7±0.01	70.0±0.1	7.0±0.01
5	71.4±0.01	85.0±0.05	17.0±0.03
10	86.8±0.01	90.0±0.1	28.0±0.02
15	90.6±0.01	95.0±0.05	49.0±0.01
20	92.7±0.01	96.7±0.1	75.0±0.02
25	93.0±0.01	97.0±0.1	80.0±0.01

API: Active pharmaceutical ingredients; DMF: Dental medicinal films.

Table 3. Calculations, based on the results of statistical processing

Time rate	Sample size	Number at the level	Number of squares	Average value at the level	Average number of square
	n_i	$Y_{i*} = \sum_{j=1}^{n_i} Y_{ij}$	$Y_{i*}^2 = \sum_{j=1}^{n_i} Y_{i*}^2$	$\overline{Y}_{i*} = \frac{1}{n_i} Y_{i*}$	$n_i(\overline{Y}_{i*})^2 = \frac{1}{n}(Y_{i*})^2$
3	6	298	14800.98	49.667	14800.67
5	6	428.3	30573.83	71.383	30573.48
10	6	520.6	45171.1	86.767	45170.73
15	6	543.5	49232.31	90.583	49232.04
20	6	556.3	51578.53	92.717	51578.28
25	6	558	51894.44	93	51894
Number	36	2904.7	243251.2		243249.2
Number by levels	$n = \sum_{i=1}^{I} n_i$	$Y_{**} = \sum_{i=1}^{I} Y_{i*}$	$Y_{**}^2 = \sum_{i=1}^{I} Y_{i*}^2$	-	$\sum_{i=1}^{I} n_i(\overline{Y}_{i*})^2$

Table 4. The comparison between the received data of release of amizon, decametoxine and chlorhexidine

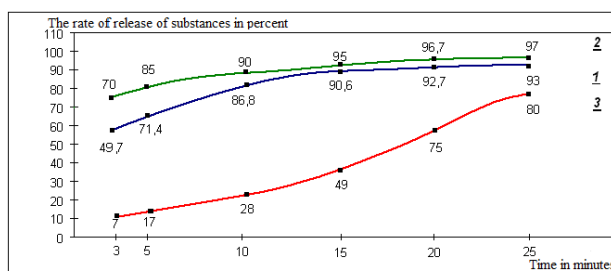| Groups	Average value at the level	25 min	20 min	15 min	10 min	5 min	3 min
		93	92.717	90.583	86.767	71.383	71.383
3	49.667	-43.333	-43.05	-40.92	-37.1	-21.716	0
5	71.383	-21.617	-21.334	-19.2	-15.384	0	21.716
10	86.767	-6.233	-5.95	-3.816	0	15.384	37.1
15	90.583	-2.417	-2.134	0	3.816	19.2	40.916
20	92.717	-0.283	0	2.134	5.95	21.334	43.05
25	93	0	0.283	2.417	6.233	21.617	43.333

Figure 1. Dynamics of active pharmaceutical ingredients release from the composition of dental medicinal films in aqueous NaCl (0.9% solution). 1: Amizon content in the tested sample (%); 2: Decametoxine content in the tested sample (%); 3: Chlorhexidine digluconate content in the tested sample (%)

Discussion

Polymeric materials and polymeric films have found a wide application in dentistry as antimicrobial and drug delivery agents due to their excellent surfaces, appropriate mechanical and biological characteristics, affordable cost, and ease in production. Oral films as carriers of antibacterial or antimycotic API for their gradual and direct release at the target surface of the oral cavity are suitable for prolonged local effects and enhancement of treatment effectiveness (Rokaya et al., 2018; Zhang et al., 2022).

Films are basically produced from a polymeric matrix which can release the API. The degradable or nondegradable films can contain a mixture of polymers, additives, API, and a solvent which can be hydrophilic or hydrophobic (Petrescu et al., 2022).

The films for dental application can be defined as hard or soft preparations of the appropriate size and shape, consisting of a matrix, in which the active substance is incorporated, or active principle, surrounded by a membrane that controls the rate of release. This DF is used in the therapy of periodontitis for incorporation into the gum pocket, as well as for applications on the gums and mucous membrane of the mouth. Depending on the composition of the polymer matrix, dissolution of such a film occurs within 1 to 30 days, and *in vitro* research showed the possibility of release of API for about 90 days (Davtyan and Golod, 2013).

Chlorhexidine is a well-known safe agent for infection control in dentistry, able to reduce plaque formation, gingival inflammation and bleeding. The search and development of various chlorhexidine containing DF for application in dental clinic is an active research area (Heling et al.,1992; McBain et al., 2003; Varoni et al., 2012; Sawada et al., 2016; Rodriguez Zorrilla et al., 2020).

PerioChip films, containing 2.5 mg chlorhexidine gluconate, are produced in Israel, on a soluble matrix, based on hydrolyzed gelatine cross linked with glutaraldehyde (Mandlik and Jha, 2007). Up to 40% of the active substance after the introduction of the film into the gum pocket is released within the first 24 hours, the rest within 7–10 days (Davtyan and Golod, 2013). Carmellose mucoadhesive oral films incorporated modified nanocomposite enriched by chlorhexidine diacetate and digluconate inhibit the growth of *Staphylococcus spp.* and *Candida spp.* (Gajdziok et al., 2015).

The performed by us current study demonstrates, that in the manufacture of dental medicinal films on a polymeric basis of hydrophilic type it is necessary to take into account the release time of each of the active pharmaceutical ingredients, in accordance with the composition of the polymeric basis. The statistical calculations on the reliability of the release of API confirm that the time of complete release of the three active substances from the polymeric base was different, which ultimately affects the time periods of pharmacological impact of the active substance in the body.

Conclusions

Due to the lack of standardization and considering individual properties of different API in DMF, a statistical analysis was performed to assess the pharmacodynamic properties of the finished products, because during the manufacture of DMF on a polymeric basis of hydrophilic type it is necessary to take into aconsideration the release time for each API.

The development of polymer-based dosage forms allows the creation of new drugs with antiseptic effects for the dentistry that have a prolonged type of release of active pharmaceutical ingredients compared to other forms of local action (ointments, gels or rinsing solutions).

Authors' contributions
IH contributed in conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, original draft preparation, review and editing, visualization, supervision, and project administration. VH contributed in conceptualization, methodology, investigation, resources, review, and editing. RL contributed in conceptualization, original draft preparation, review, and editing.

Ethical considerations
Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

References

Anistratenko VO, Fedorov VH. Mathematical planning of experiments in agroindustrial complex [in Ukrainian]. Kyiv: Vyshcha Shkola Publishing House, 1993; 374.

Bakhrushyn VY. Investigation of the properties of dispersion degree evaluation, obtained by the group method. [in Ukrainian]. Journal of Complex Systems and Processes. 2006; 1:3-7.

Davtyan LL, Golod AS. Use of polymers for composition of new medicines in the film form [in Ukrainian]. Farmatsevtychnyi zhurnal. 2013;5:51-7.

Gajdziok J, Holešová S, Štembírek J, Pazdziora E, Landová H, Doležel P, et al. Carmellose mucoadhesive oral films containing vermiculite/chlorhexidine nanocomposites as innovative biomaterials for treatment of oral infections. BioMed Research International. 2015; 580146. doi: 10.1155/2015/580146

Gasmi Benahmed A, Gasmi A, Arshad M, Shanaida M, Lysiuk R, Peana M, Pshyk-Titko I, Adamiv S, Shanaida Y, Bjorklund G. Health benefits of xylitol. Appl Microbiol Biotechnol. 2020;104(17):7225-7237. doi: 10.1007/s00253-020-10708-7

Gasmi Benahmed A, Gasmi A, Menzel A, Hrynovets I, Chirumbolo S, Shanaida M, Lysiuk R, Shanaida Y, Dadar M, Bjorklund G. A review on natural teeth whitening. J Oral Biosci. 2022;64(1):49-58. doi: 10.1016/j.job.2021.12.002

Gasmi Benahmed A, Gasmi A, Tippairote T, Mujawdiya PK, Avdeev O, Shanaida Y, Bjørklund G. Metabolic Conditions and Peri-Implantitis. Antibiotics (Basel). 2022; 29; 12(1):65. doi: 10.3390/antibiotics12010065

Heling I, Sommer M, Steinberg D, Friedman M, Sela MN. Microbiological evaluation of the efficacy of chlorhexidine in a sustained-release device for dentine sterilization. Int Endod J. 1992;25(1):15-9. doi: 10.1111/j.1365-2591.1992.tb00943.x

Herasymenko SS, Golovach AV, Yerina AM et al. Statistics: textbook [in Ukrainian]. Kyiv: KNEU, 2000; 467.

Horoneskul MM. Tables of functions and critical points of distributions. Sections: Probability Theory. Mathematical statistics. Mathematical methods in psychology [in Ukrainian]. Kharkiv: NUSDU, 2009; 90.

Hrynovets IS. Development of the composition of technology and research of dental medicinal films (PhD thesis) [in Ukrainian]. Lviv, Danylo Halytsky Lviv National Medical University, 2013; 155.

Katakura A. Necessity of providing dental care to medically compromised patients in Japan's super-aged society and the way forward. Jpn Dent Sci Rev. 2022; 58: 279-285. doi: 10.1016/j.jdsr.2022.09.002

Kida D, Zakrzewska A, Zborowski J, Szulc M, Karolewicz B. Polymer-Based Carriers in Dental Local Healing-Review and Future Challenges. Materials (Basel). 2021; 14;14(14): 3948. doi: 10.3390/ma1414394

Lamster IB, Eaves K. A model for dental practice in the 21st century. Am J Public Health. 2011; 101(10): 1825-30. doi: 10.2105/AJPH.2011.300234

Mandil O, Sabri H, Manouchehri N, Mostafa D, Wang HL. Root coverage with apical tunnel approach using propolis as a root conditioning agent: A case report with 2- year follow- up and review of the literature. Clinical and Experimental Dental Research. 2023 Jun 20. https://doi.org/10.1002/cre2.751

Mandil O, Sabri H, Manouchehri N, Mostafa D, Wang HL. Root coverage with apical tunnel approach using propolis as a root conditioning agent: A case report with 2- year follow- up and review of the literature. Clinical and Experimental Dental Research. 2023 Jun 20. https://doi.org/10.1002/cre2.751

Mandlik VB, Jha AK. Periochip. Med J Armed Forces India. 2007;63(4):368-9. doi: 10.1016/S0377-1237(07)80019-0

Manouchehri N, Ghodsi S, Atri F, Sarraf P, Seyedi D, Valizadeh S. Effect of pretreatment of root dentin surface with cold atmospheric plasma on improving the bond strength of fiber post and resin cement: In vitro study. Clinical and Experimental Dental Research. 2023 Jun 8. https://doi.org/10.1002/cre2.744

Manouchehri N. A review of the most widely used medicinal plants in the treatment of dental and oral disorders and diseases. Journal of Biochemicals and Phytomedicine. 2022; 1(1): 3–7. doi: 10.34172/jbp.2022.2

McBain AJ, Bartolo RG, Catrenich CE, Charbonneau D, Ledder RG, Gilbert P. Effects of a chlorhexidine gluconate-containing mouthwash on the vitality and antimicrobial susceptibility of in vitro oral bacterial ecosystems. Appl Environ Microbiol. 2003;69(8):4770-6. doi: 10.1128/AEM.69.8.4770-4776.2003

Petrescu N, Crisan B, Aghiorghiesei O, Sarosi C, Mirica IC, Lucaciu O, Iu?an SAL, Dirzu N, Apostu D. Gradual Drug Release Membranes and Films Used for the Treatment of Periodontal Disease. Membranes (Basel). 2022; 17;12(9):895. doi: 10.3390/membranes12090895

Rodriguez Zorrilla S, Blanco Carrion A, Garcia Garcia A, Galindo Moreno P, Marichalar Mendia X, Seoane Prado R, Perez Estevez AJ, Perez-Sayans M. Effect of antiseptic gels in the microbiologic colonization of the suture threads after oral surgery. Sci Rep. 2020; 20;10(1):8360. doi: 10.1038/s41598-020-65007-y

Rokaya D, Srimaneepong V, Sapkota J, Qin J, Siraleartmukul K, Siriwongrungson V. Polymeric materials and films in dentistry: An overview. J Adv Res. 2018; 3;14:25-34. doi: 10.1016/j.jare.2018.05.001

Sanders KA, Zerden LS, Zomorodi M, Ciarrocca K, Schmitz KL. Promoting Whole Health in the Dental Setting: Steps Toward an Integrated Interprofessional Clinical Learning Environment Involving Pharmacy, Social Work, and Nursing. Int J Integr Care. 2021; 18;21(4):20. doi: 10.5334/ijic.5814

Santacroce L, Spirito F, Bottalico L, Muzio EL, Charitos IA, Potenza MA, Montagnani M, Jirillo E. Current Issues and Perspectives in Antimicrobials use in Dental Practice. Curr Pharm Des. 2022; 28(35):2879-2889. doi: 10.2174/1381612828666220920114723

Sawada K, Fujioka-Kobayashi M, Kobayashi E, Schaller B, Miron RJ. Effects of Antiseptic Solutions Commonly Used in Dentistry on Bone Viability, Bone Morphology, and Release of Growth Factors. J Oral Maxillofac Surg. 2016;74(2):247-54. doi: 10.1016/j.joms.2015.09.029

Varoni E, Tarce M, Lodi G, Carrassi A. Chlorhexidine (CHX) in dentistry: state of the art. Minerva Stomatol. 2012;61(9):399-419.

Vasylenko OA, Sencha IA. Mathematical and statistical methods of analysis in applied research: textbook [in Ukrainian]. Odessa: O.S. Popov ONAT, 2011; 166.

Wang Z, Shen Y, Haapasalo M. Dental materials with

antibiofilm properties. Dent Mater. 2014;30(2):e1-16. doi: 10.1016/j.dental.2013.12.001

Zhang Y, Jiang R, Lei L, Yang Y, Hu T. Drug delivery systems for oral disease applications. J Appl Oral Sci. 2022; 9;30: 20210349. doi: 10.1590/1678-7757-2021-0349.

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