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Local anaesthetics classified using chemical structural indicators

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ABSTRACT

A group of 28 local anaesthetics is classified into a periodic table by using an algorithm based on the theory of entropy information. Five features in hierarchical order are used to classify structurally the anaesthetics. From these features, the first three mark the group or column, while the two last mark the row or period in the table. Those anaesthetics in the same group and period have maximum similarity in properties. Furthermore, those with only the same group present important similarity.

KEYWORDS: *Periodic property, Periodic law, Classification, Information entropy, Equipartition conjecture, Procaine analogue.*

RESUMEN

Un grupo de 28 anestésicos locales se clasifica en una tabla periódica utilizando un algoritmo basado en la entropía de la teoría de la información. Para clasificar estructuralmente los anestésicos se emplean cinco características en orden jerárquico. De estas características, las tres primeras marcan el grupo o columna de la tabla, mientras que las dos últimas marcan la fila o período. Los anestésicos de un mismo grupo y período presentan semejanza máxima en sus propiedades.

PALABRAS CLAVE: *Propiedad periódica, Ley periódica, Clasificación, Entropía de la información, Conjetura de Equipartición, Análogo de la procaína.*

INTRODUCTION

With the emergence of new laser and surgical techniques, the need for more effective topical anaesthesia continues to grow. There are now several topical local anaesthetics that are being used before various dermatologic procedures. EMLA is the most commonly used agent, however, several new topical anaesthetics have been released recently that claim increased efficacy and faster onset of action. Topical anaesthetics are weak bases typically constructed of three important components: an aromatic ring, an intermediate-length ester or amide linkage, and a tertiary amine. The ester anaesthetics have an ester linkage, while the amide anaesthetics have an amide linkage between the aromatic ring and intermediate chain. Ester-type topical anaesthetics are metabolized by plasma cholinesterase and other non-specific esterases, while amide anaesthetics are primarily metabolized in the liver *via* microsomal enzymes. Allergic contact reactions to the ester group of anaesthetics are common, while amide anaesthetics, including lidocaine and prilocaine, are rare sensitizers [27]. The metabolite para-aminobenzoic acid (PABA) formed by ester hydrolysis is capable of causing allergic reactions in a small percentage of patients [22]. Ester-linked anaesthetics are contraindicated in patients with allergies to PABA, hair dyes and sulphonamides. Topical anaesthetics prevent the initiation and transmission of nerve impulses and provide cutaneous analgesia by targeting free nerve endings in the dermis. Topical anaesthetics block nerve impulse conduction by interfering with the function of sodium channels. By inhibiting sodium flux, the threshold for nerve excitation increases until the ability to generate an action potential is lost. The *stratum corneum* is the main barrier to topical anaesthetic delivery [2]. The aromatic portion is primarily responsible for the lipid solubility that allows diffusion across the nerve cell membrane, determining the intrinsic potency of these agents (*cf.* Fig. 1) [4–6]. Both the aromatic and amine portion determine protein-binding characteristics, which are felt to be the primary determinant of anaesthesia duration.

Different methods for evaluating and comparing anaesthetic efficacy have included venipuncture, pinprick testing, split-thickness skin graft donation and laser pulses as pain stimuli. Laser-induced thermal pain stimuli are advantageous for comparing topical anaesthetics by providing reproducible,

quantifiable stimuli with minimal intraindividual variation. Laser pulses also provide selective activation of nociceptors, without interference from mechanosensitive receptors. The drugs exist in both positively charged and uncharged forms under normal in vivo conditions. The most important clinical properties of local anaesthetics are potency, onset, duration of action as well as relative blockade of sensory and motor fibres. These qualities are related primarily to the physicochemical properties of the various compounds. In general, lipid solubility determines the relative intrinsic potency of the various agents, while protein binding influences the duration of anaesthesia and pKa is correlated with the onset of action. The local anaesthetics for infiltration, peripheral nerve blockade and extradural anaesthesia can be classified into three groups: agents of low potency and short duration (procaine, 2-chloroprocaine), agents of moderate potency and duration (lidocaine, mepivacaine, prilocaine), as well as agents of high potency and long duration (tetracaine, bupivacaine, etidocaine, S-ropivacaine). These local anaesthetics also vary in terms of onset: 2-chloroprocaine, etidocaine, lidocaine, mepivacaine and prilocaine have a rapid onset, while procaine, tetracaine and bupivacaine are characterized by a longer latency period; bupivacaine presents a moderate onset. General anaesthetics, including inhaled agents, e.g., halothane, isoflurane and desflurane, as well as intravenous agents, e.g., propofol, are now generally considered to act by modulating the effects of γ-aminobutyric acid (GABA) and type A (GABAA) receptors and/or by affecting other ion channels [5,20]. General anaesthetics that do not act at GABAA receptors are NO2, Xe and ketamine. The theory that inhaled anaesthetics probably dissolve in the lipid-rich neuronal cell membranes near the nerve endings and changes their volume or fluidity is not well supported by experimental evidence. For example, such theories fail to account for differences in potency for stereoisomers of general anaesthetics [11]. Local anaesthetics act by blocking voltage-gated Na+ channels although some agents, e.g., propranolol, affect other proteins as well.

A simple computerized algorithm useful for establishing a relationship between chemical structures and their biological activities or significance is proposed and exemplified here (cf. Reference [27] or [3] for a review). The starting point is to use an informational or configurational entropy for pattern recognition purposes. This entropy is formulated on the basis of a matrix of similarity between two chemical or biochemical species. The presented example shows a classification of local anaesthetics on the basis of their similarity with procaine [1]. As entropy is weakly discriminating for classification purposes, the more powerful concept of entropy production and its equipartition conjecture are introduced [28]. In earlier publications, the periodic classification of local anaesthetics (procaine analogues) is analyzed [29,30]. The aim of the present report is to develop the learning potentialities of the code and, since molecules are more naturally described via a varying size structured representation, the study of general approaches to the processing of structured information. The following section presents the computational method. Next section describes the classification algorithm. Following that, the equipartition conjecture of entropy production is exposed. Next section analyzes the learning procedure. Then a section describes program MolClas. A new section explains program GraphCor. Next presents and discusses the calculation results. Last summarizes the conclusions.

COMPUTATIONAL METHOD

The key problem in classification studies is to define similarity indices when several criteria of comparison are involved. The first step in quantifying the concept of similarity for molecules of local anaesthetics is to list the most important portions of such molecules. Furthermore, the vector of properties $\bar{i}$ = <i1,i2,…ik,…> should be associated to each local anaesthetic i, whose components correspond to different characteristic groups of the molecule of anaesthetic, in a hierarchical order according to the expected importance of their pharmacological potency. If the m-th portion of the molecule is pharmacologically more significant for the anaesthetic effect than the k-th portion, then m < k. The components ik are "1" or "0" according to whether a similar (or identical) portion of rank k is present or absent in anaesthetic i compared with the reference anaesthetic. Our analysis includes such chemical compounds that fit the following general scheme: (lipophilic portion)–(intermediate chain)–(hydrophilic portion), since these are the most numerous and have the widest range of uses among the species used in practice of local anaesthesia [22]. The lipophilic portion normally consists of at least one phenyl radical, the hydrophilic portion is most often a secondary or tertiary amine, and the intermediate chain commonly has an ester or amide linkage [6]. It is assumed that the structural elements of a local anaesthetic molecule can be ranked, according to their contribution to anaesthetic potency, in the following order of decreasing importance: lipophilic portion > hydrophilic portion > intermediate chain > number of nitrogen atoms > number of oxygen atoms. The lipophilic portion is primarily responsible for the lipid solubility that allows diffusion across the nerve cell membrane, determining the intrinsic potency of local anaesthetics. Both the lipophilic and hydrophilic portions determine protein-binding characteristics, which are felt to be the primary determinant of anaesthesia duration. Procaine or novocaine {4-aminobenzoic acid [2-(diethylamino)ethyl] ester, 4-H2NC6H4CO2[CH2CH2N(C2H5)2], PR} is a tertiary amine, containing a primary amino group linked to an aromatic ring (cf. Fig. 2). Consequently, it may exist as a neutral molecule (PR), a monocation (4-H2NC6H4CO2[CH2CH2NH+(C2H5)2], PRH+), or a dication (4-H2NH+C6H4CO2[CH2CH2NH+(C2H5)2], PRH22+). In procaine, the lipophilic portion is a phenyl radical, the hydrophilic portion is an amine, the intermediate chain is an ester, there are two N atoms and two O atoms; obviously, its associated vector is <11111>. In this work, procaine was selected as a reference anaesthetic because it was the first synthetic compound successfully used for regional anaesthesia and, in this and most other local anaesthetics, the lipophilic portion consists of at least one phenyl radical, the hydrophilic portion is a secondary or tertiary amine, and/or the intermediate chain has an ester linkage. This improves the quality of the classification for those anaesthetics similar to procaine. The selection as reference of an anaesthetic dissimilar to procaine, e.g., dibucaine, would not improve the quality of the classification for those anaesthetics similar to procaine. Furthermore, Covino results included both ester (similar to procaine) and amide (similar to prilocaine) anaesthetics; the inclusion of the results in the classification described below improves the quality of the taxonomy, for both types of anaesthetics.

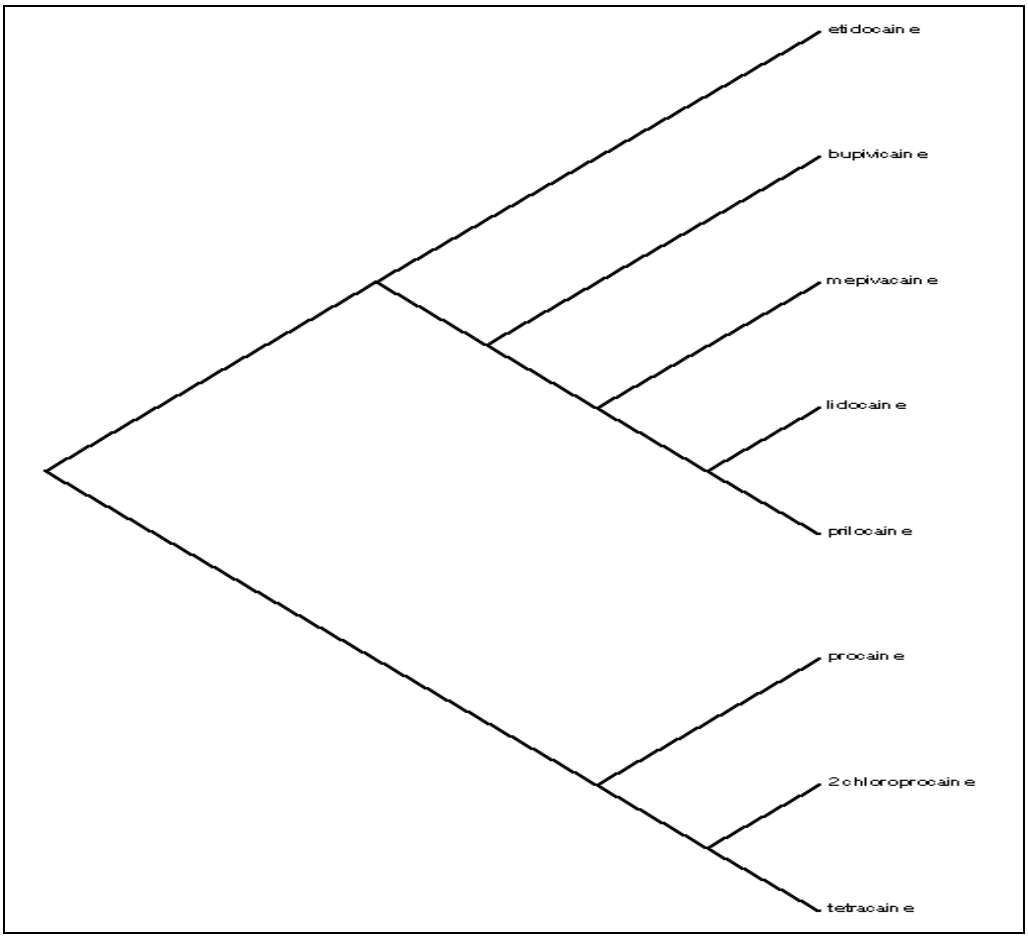


Figure 1. Anaesthetics by chemical structure/physicochemical properties/pharmacological properties.

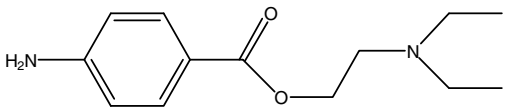


Figure 2. Molecular structure of a local anaesthetic procaine neutral molecule.

Table 1 contains the vectors associated to 28 local anaesthetics. The <11110> vector is associated to benoxinate (Table 1), since there are three O atoms in this case. The <10101> vector is associated to benzocaine since the hydrophilic partition is not an amine, and there is one N atom in this case. Let us denote by rij ($0 \leq rij \leq 1$) the similarity index of two anaesthetics associated to the $\bar{i}$ and $\bar{j}$ vectors, respectively. The relation of similitude is characterized by a similarity matrix $\overline{\overline{R}} = [r_{ij}]$. The similarity index between two anaesthetics $\bar{i}$ =<i1, i2,… ik,…> and $\bar{j}$ =<j1, j2,… jk,…> is defined as:

$$r_{ij} = \sum_k t_k \left(a_k\right)^k \quad (k = 1, 2, \ldots) \tag{1}$$

where $0 \leq ak \leq 1$ and tk = 1 if ik = jk, but tk = 0 if ik ≠ jk. This definition assigns a weight (ak)k to any property involved in the description of molecules i or j.

CLASSIFICATION ALGORITHM

The grouping algorithm uses the stabilized matrix of similarity, obtained by applying the max-min composition rule o defined by:

$$\left(\overline{\overline{R}} \mathrm{o} \overline{\overline{S}}\right)_{ij} = \max_k \left[\min_k \left(r_{ik}, s_{kj}\right)\right] \tag{2}$$

where $\overline{\overline{R}} = \left[r_{ij}\right]$ and $\overline{\overline{S}} = \left[s_{ij}\right]$ are matrices of the same type, and $\left(\overline{\overline{R}} \mathrm{o} \overline{\overline{S}}\right)_{ij}$ is the (i,j)-th element of the matrix $\overline{\overline{R}}$ o $\overline{\overline{S}}$ [18]. It can be shown that when applying this rule iteratively so that $\overline{\overline{R}}(n+1) = \overline{\overline{R}}(n) \mathrm{o} \overline{\overline{R}}$, there exists an integer n such that: $\overline{\overline{R}}(n) = \overline{\overline{R}}(n+1) = \ldots$ The resulting matrix $\overline{\overline{R}}(n)$ is called the stabilized similarity matrix. The importance of stabilization lies in the fact that in the classification process, it will generate a partition in disjoint classes. From now on, it is understood that the stabilized matrix is used and designated by $\overline{\overline{R}}(n) = \left[r_{ij}(n)\right]$. The grouping rule is the following: i and j are assigned to the same class if rij(n) ≥ b. The class of i noted $\hat{i}$ is the set of species j that satisfies the rule rij(n) ≥ b. The matrix of classes is:

$$\overline{\overline{\hat{R}}}(n) = \left[\hat{r}_{\hat{i}\hat{j}}\right] = \max_{s,t}\left(r_{st}\right) \quad (s \in \hat{i},\ t \in \hat{j}) \tag{3}$$

where s stands for any index of a species belonging to the class $\hat{i}$ (similarly for t and $\hat{j}$). Rule (3) means finding the largest similarity index between species of two different classes.

In information theory, the information entropy h measures the surprise that the source emitting the sequences can give [19,20]. For a single event occurring with probability p, the degree of surprise is proportional to –ln p. Generalizing the result to a random variable X (which can take N possible values x1, …, xN with probabilities p1, …, pN), the average surprise received on learning the value of X is – pi ln pi. The information entropy associated with the matrix of similarity $\overline{\overline{R}}$ is:

$$h\left(\overline{\overline{R}}\right) = -\sum_{i,j} r_{ij} \ln r_{ij} - \sum_{i,j} \left(1 - r_{ij}\right) \ln\left(1 - r_{ij}\right) \tag{4}$$

Denote also by Cb the set of classes and by $\overline{\overline{R}}_b$ the matrix of similarity at the grouping level b. The information entropy satisfies the following properties.

1. $h\left(\overline{\overline{R}}\right) = 0$ if rij = 0 or rij = 1.
2. $h\left(\overline{\overline{R}}\right)$ is maximum if rij = 0.5, i.e., when the imprecision is maximum.
3. $h\left(\overline{\overline{R}}_b\right) \le h\left(\overline{\overline{R}}\right)$ for any b, i.e., classification leads to a loss of entropy.
4. $h\left(\overline{\overline{R}}_{b_1}\right) \le h\left(\overline{\overline{R}}_{b_2}\right)$ if b1 < b2, i.e., the entropy is a monotone function of the grouping level b.

THE EQUIPARTITION CONJECTURE OF ENTROPY PRODUCTION

In the classification algorithm, each hierarchical tree corresponds to a dependence of entropy on the grouping level, and thus an h–b diagram can be obtained. The Tondeur and Kvaalen equipartition conjecture of entropy production is proposed as a selection criterion among different variants resulting from classification among hierarchical trees. According to this conjecture, for a given charge or duty, the best configuration of a flowsheet is the one in which entropy production is most uniformly distributed, i.e., closest to a kind of equipartition. One proceeds here by analogy using information entropy instead of thermodynamic entropy. Equipartition implies a linear dependence, that is a constant production of entropy along the b scale, so that the equipartition line is described by:

$$h_{\mathrm{eqp}} = h_{\max}\ b \tag{5}$$

Indeed, since the classification is discrete, a realistic way of expressing equipartition would be a regular staircase function. The best variant is chosen to be that minimizing the sum of squares of the deviations:

$$SS = \sum_{b_i} \left(h - h_{\mathrm{eqp}}\right)^2 \tag{6}$$

Table 1. Vector properties of local anaesthetics analogues of procaine.

1. benoxinate	<11110>	15. Lidocaine	<11010>
2. benzocaine	<10101>	16. Mepivacainebb	<11010>
3. bupivacaine	<11010>	17. Piperocaine	<11101>
4. butacaine	<11111>	18. Pramoxine	<11000>
5. butamben	<10101>	19.prilocaine	<11010>
6. 2-chloroprocaine	<11111>	20. Procaine	<11111>
7. cocaine	<11100>	21. Proparacaine	<11110>
8. cyclomethycaine	<11100>	22. Propoxycaine	<11110>
9. dibucaine	<01001>	23. Tetracaine	<11111>
10. dimethisoquin	<01010>	24. Tocainide	<11010>
11. diperodon	<11000>	25. Mexiletine	<11000>
12. dyclonine	<11001>	26. Propanolol	<01001>
13. etidocaine	<11010>	27. Phenytoin	<10011>
14. hexylcaine	<11101>	28. S-ropivacaine	<11010>

LEARNING PROCEDURE

Learning procedures similar to those encountered in stochastic methods are implemented as follows [21]. Consider a given partition in classes as good or ideal from practical or empirical observations. This corresponds to a reference similarity matrix $\overline{\overline{S}} = \left[s_{ij}\right]$ obtained for equal weights a1 = a2 = … = a and for an arbitrary number of fictious properties. Next consider the same set of species as in the good classification and the actual properties. The similarity degree rij is then computed with Equation (1) giving the matrix $\overline{\overline{R}}$. The number of properties for $\overline{\overline{R}}$ and $\overline{\overline{S}}$ may differ. The learning procedure consists in trying to find classification results for $\overline{\overline{R}}$ as close as possible to the good classification. The first weight a1 is taken constant and only the following weights a2, a3,… are subjected to random variations. A new similarity matrix is obtained using Equation (1) and the new weights. The distance between the partitions in classes characterized by $\overline{\overline{R}}$ and $\overline{\overline{S}}$ is given by:

$$D = -\sum_{ij} \left(1 - r_{ij}\right) \ln \frac{1 - r_{ij}}{1 - s_{ij}} - \sum_{ij} r_{ij} \ln \frac{r_{ij}}{s_{ij}} \qquad \forall\ 0 \le r_{ij}, s_{ij} \le 1 \tag{7}$$

The result of the algorithm is a set of weights allowing adequate classification. Such a procedure has been applied in the synthesis of complex flowsheets using of information entropy [2].

DESCRIPTION OF CALCULATION PROGRAM MOLCLAS

We have written program MolClas for molecular classification based on the equipartition conjecture of entropy production. MolClas allows reading the coefficients from input and optimizing them. MolClas allows either calculating the correlation matrix or reading it from input. An option has been included to transform negative correlation coefficients to zero. The program punches both similarity and difference matrices, as well the latter in format NEXUS (.NEX) for programs PAUP, MacClade and SplitsTree. MolClas performs both single- and complete linkage hierarchical cluster analyses (CAs) of the compounds by using the IMSL subroutine CLINK [26].

In our study of local anaesthetics, negative correlation coefficients are taken as zero, because they are based on structural-molecular differences.

DESCRIPTION OF GRAPHIC PROGRAM GRAPHCOR

We have written graphic program GraphCor for performing the graphical correlation diagram. GraphCor can present high partial correlations ($|r| \geq 0.75$) in red, medium partial correlations ($0.50 \leq |r| < 0.75$) in orange, low partial correlations ($0.25 \leq |r| < 0.50$) in yellow and zero partial correlations ($|r| < 0.25$) in white. An option has been included to transform negative correlation coefficients to zero.

For local anaesthetics, negative correlation coefficients are taken as zero because they are based on structural differences.

MolClas and GraphCor are available form the authors at Internet (francisco.torrens@uv.es) and are free for academics.

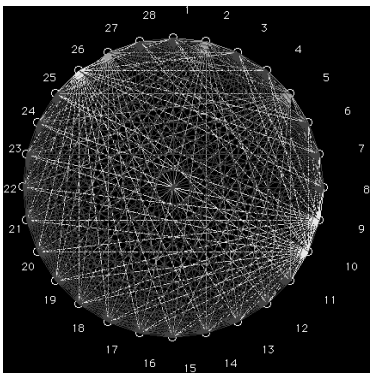


Figure. 3. Partial correlation diagram: High, medium and low correlations in greyscale.

CALCULATION RESULTS AND DISCUSSION

In the present report 28 local anaesthetics analogues of procaine (Table 1) have been studied. The analysis includes such chemical compounds that fit the following general scheme: lipophilic portion–intermediate chain–hydrophilic portion, since among the species used in practice of local anaesthesia, these are the most numerous and have the widest range of uses. The lipophilic portion normally consists of at least one phenyl radical; the hydrophilic portion is most often a secondary or tertiary amine; the intermediate chain commonly has an ester or amide linkage. The matrix of Pearson correlation coefficients between each pair of vector properties <i1,i2,i3,i4,i5> of the 28 anaesthetics has been calculated. The Pearson intercorrelations are illustrated in the partial correlation diagram, which contains 133 high partial correlations ($r \geq 0.75$), 76 medium partial correlations ($0.50 \leq r < 0.75$) and 22 low partial correlations ($0.25 \leq r < 0.50$). Pairs of anaesthetics with high partial correlations show a similar vector property. However, the results should be taken with care, because the four compounds with constant <11111> vector (Entries 4, 6, 20 and 23 in Table 1) show null standard deviation, causing high partial correlations ($r = 1$) with any local anaesthetic, which is an artifact. With the equipartition conjecture, the intercorrelations are illustrated in the partial correlation diagram, which contains 237 high, 66 medium and 66 low partial correlations (cf. Fig. 3). Notice that 24 of the 108 (4×6/27) high partial correlations of Entries 4, 6, 20 and 23 have been corrected; e.g., for Entry 4, the correlations with Entries 2, 5 and 27 are medium, as well as its correlations with Entries 9, 10 and 26 are low partial correlations.

The grouping rule in the case with equal weights ak = 0.5, for $0.94 \leq b1 \leq 0.96$ allows the classes:

C_{b_1} = (1,21,22)(2,5)(3,13,15,16,19,24,28)(4,6,20,23)(7,8)(9,26)(10)(11,18,25)(12)(14,17)(27)

The 11 classes are obtained with the associated entropy $h\left(\overline{\overline{R}}_{b_1}\right)$ = 58.86. Both dendrogram (binary tree) and radial tree [24,25] matching to <i1,i2,i3,i4,i5> and C_{b_1} are calculated [26]; they provide a binary taxonomy of Table 1, which separates the same 11 classes. In particular, the ester (benzocaine, 2-chloroprocaine, procaine, tetracaine) and amide local anaesthetics (bupivacaine, dibucaine, etidocaine, lidocaine, mepivacaine, prilocaine, S-ropivacaine) are always grouped in different classes. The agents of low potency and short duration (procaine, 2-chloroprocaine) are separated from the agents of high potency and long duration (bupivacaine, etidocaine, S-ropivacaine), while the agents of moderate potency and duration (mepivacaine, prilocaine) are classified together with the latter.

Those anaesthetics belonging to the same class appear higly correlated in both partial correlation diagrams (Fig. 3), in agreement with previous results (Fig. 1).

Table 2. Classification level, number of classes and entropy for the local anaesthetics.

Classification level b	Number of classes	Entropy h
1.00	28	362.02
0.96	11	59.65
0.93	8	31.31
0.87	5	12.00
0.78	4	7.23
0.75	3	3.95
0.56	2	1.66
0.25	1	0.14

At level b2 with $0.85 \leq b2 \leq 0.87$ the set of classes is

C_{b_2} = (1,4,6,7,8,14,17,20,21,22,23)(2,5)(3,11,12,13,15,16,18,19,24,25,28)(9,10,26)(27)

Five classes result in this case; the entropy is $h\left(\overline{\overline{R}}_{b_2}\right)$ = 12.20. Both dendrogram and radial tree matching to <i1,i2,i3,i4,i5> and C_{b_2} separate the same five classes, in agreement with both partial correlation diagrams, dendrogram, binary tree and previous results (Fig. 1-3). A high degree of similarity is found for Entries 9 and 26, i.e., dibucaine and propanolol, as well as Entries 2 and 5, i.e., benzocaine and butamben. Again, the ester and amide local anaesthetics are grouped in different classes; the agents of low potency and short duration are separated from the agents of high–medium potency and long–medium duration. The lower level b2 classification process shows lower entropy and, therefore, may be more parsimonious. The classification model divides the point process into two components, viz. signal, and noise; the lower-level b2 may have greater signal-to-noise ratio than the higher-level b1 classification process. Naturally, Entries 4, 6, 20 and 23, i.e., butacaine, 2-chloroprocaine, procaine and tetracaine, belong to the same class at any grouping level b, except at the highest level above in which each class contains only one species. A detailed classification at level b1 into 11 classes, and a less detailed classification at a lower level b2 into five classes can be selected, taking into account the amount of entropy variation. A comparative analysis of the set containing 1–11 classes is summarized in Table 2, in agreement with previous results (Fig. 1). From the set containing 1–11 classes (Table 2), the radial tree matching to <i1,i2,i3,i4,i5> and $C_{b_{1-11}}$ separates the same five and 11 classes, in agreement with both partial correlation diagrams, dendrograms, binary trees and previous results (Fig. 1-3). Once more, the ester and amide local anaesthetics are grouped in different classes; the agents of low potency and short duration are separated from the agents of high–medium potency and long–medium duration. The resulting partition into classes compares well with other from Covino considered as good. He compared three ester, viz. 2-chloroprocaine, procaine, and tetracaine, as well as five amide local anaesthetics, viz. bupivacaine, etidocaine, lidocaine, mepivacaine, and prilocaine, based on chemical configuration (aromatic lipophilic group, intermediate chain and amine hydrophilic group), four physicochemical properties (molecular weight, pKa, partition coefficient and protein binding), as well as three pharmacological properties (onset, relative potency and duration). The onset is determined primarily by pKa. The percentage of a local anaesthetic that is present in the neutral form, when injected to tissue of pH 7.4, decreases with pKa, according to the equation of Henderson–Hasselbalch: pH = pKa + log ([PR]/[HPR+]). The potency is determined primarily by lipid solubility, which increases with partition coefficient. Both lipid solubility and partition coefficient are mainly due to the neutral forms. Different conformations have different partition coefficients, lipid solubilities and potencies. It would be of interest to study the effect of different intermediate chain lengths. In particular, the presence of a double bond in a chain would increase rigidity and enhance potency; e.g., the conjugated enol group in 3-phenyl-2-propen-1-ol determines a greater membrane permeability, with respect to 3-phenyl-1-propanol.

The inclusion of this comparison in the dendrogram of the present work (cf. Fig. 4) is in agreement with both partial correlation diagrams, dendrograms, binary trees and previous results (Fig. 1-3). The classification scheme from 1–11 levels is conserved after the addition of Entry 28 (S-ropivacaine). Fawcett et al. compared S-bupivacaine with racemic bupivacaine [27]. S-ropivacaine is structurally close to bupivacaine; the main difference is that the former is a pure S-(–) enantiomer where the latter is a racemate. One more time, the ester and amide local anaesthetics are grouped in different classes; the agents of low potency and short duration are separated from the agents of high–medium potency and long–medium duration. Moreover, the classification presents lower bias and greater precision, resulting in lower divergence with respect to the original distribution. Therefore, the approach is quite general. However, the inclusion of other local anaesthetics could change the detail, i.e., subsequent classifications with more than 11 levels. A natural trend is to interchange similar anaesthetics in the composition of complex drugs, e.g., the eutectic mixture of local anaesthetics (EMLA®, lidocaine–prilocaine 2.5/2.5% w [4]). However, mixtures of dissimilar anaesthetics are also used, e.g., betacaine-LA (lidocaine–prilocaine–dibucaine) [14] and S-caine (1:1 lidocaine–tetracaine eutectic mixture) [15].

A set of geometrical descriptors for the local anaesthetics (cf. Table 3) has been calculated with our program TOPO [31] and our version of program GEPOL [24]. This comparison is in agreement with both partial correlation diagrams, dendrograms, binary trees and previous results (Fig. 1, 3 and 4).

A set of topological indices for the local anaesthetics (cf. Table 4) has been calculated with TOPO and GEPOL. This comparison is in agreement with both partial correlation diagrams, dendrograms, binary trees and previous results (Fig.1, 3 and 4, and Table 3).

The predictions for topical anaesthetics and ice, both not included in the models, are included in Table 5. The predictions have been compared with experimental results. The relative potency is obtained from the mean pain scores after application of topical anaesthetics for 60 minutes. ELA-max is superior to tetracaine and betacaine-LA at 60 minutes, while EMLA® is superior to betacaine-LA at 60 minutes, which is in partial agreement with our prediction. The relative potency after removal is obtained from the mean pain scores 30 minutes after removal of the topical anaesthetics. ELA-max and EMLA® are superior to tetracaine and betacaine-LA 30 minutes after the 60-minute application period, which is in partial agreement with our prediction. Increased anaesthetic benefit is obtained 30 minutes after removal, which suggests that a reservoir of anaesthetic is located and stored in the upper skin layers during application, providing additional anaesthetic benefit after removal. Although EMLA® is more potent than ice, ice has advantages in easy of use, fast action and is less expensive than EMLA® [10].

Table 3. Geometrical descriptors for local anaesthetics.

Figure 4. Dendrogram for anaesthetics including physicochemical and pharmacological properties

Table 3. Geometrical descriptors for local anaesthetics

	Va	V ref.b	Sc	S ref.b	ASd	AS ref.b	HBASe	HLASf	AS'g	AS' ref.b
Benoxinate	298.5	300.9	361.69	380.97	586.7	601.2	488.5	98.2	1103.7	1115.6
Benzocaine	154.9	156.1	192.40	200.27	353.1	359.3	247.8	105.3	737.2	743.2
Bupivacaine	287.1	289.5	333.55	351.66	523.8	537.6	436.2	87.6	974.2	984.9
2-Chloroprocaine	244.8	246.6	292.53	308.78	478.3	487.6	324.0	154.3	923.1	932.6
Cocaine	262.6	264.8	305.85	321.87	483.7	495.9	429.9	53.8	908.5	917.3
Dibucaine	326.2	329.0	384.48	405.00	620.8	636.4	558.7	62.1	1149.2	1161.4
Dyclonine	277.9	280.1	332.33	349.34	555.1	568.1	516.1	39.0	1058.9	1070.2
Lidocaine	230.6	232.5	265.92	281.80	428.5	438.2	395.6	32.9	838.5	846.7
Phenytoin	219.1	220.7	245.93	259.04	414.8	423.8	297.3	117.5	825.2	833.7
Prilocaine	219.3	221.1	259.56	273.23	440.5	450.3	387.7	52.8	861.9	870.8
Procaine	229.0	230.9	276.72	292.74	465.4	475.1	354.2	111.2	909.6	919.7
(R)-Propanolol	248.0	249.9	289.07	305.79	469.5	480.7	415.9	53.6	900.9	909.0
(S)-Propanolol	247.6	249.5	291.77	306.41	486.9	497.4	429.3	57.6	942.6	950.9
Tetracaine	256.6	258.5	311.98	329.21	528.6	541.9	462.9	65.7	1018.4	1028.3

a Molecular volume (Å3).
b Reference: calculations carried out with the GEPOL program.
c Molecular surface area (Å2).
d Water accessible surface area (Å2).
e Hydrophobic accessible surface area (Å2).
f Hydrophilic accessible surface area (Å2).
g Side-chain accessible surface area (Å2)

Table 4. Topological indices for local anaesthetics.

Alcoholation+Molecule	Ga	G ref.b	G'c	G' ref.b	Dd	D ref.b	D'e
Benoxinate	0.597	0.570	1.212	1.266	1.385	1.400	1.518
Benzocaine	0.725	0.700	1.242	1.283	1.286	1.295	1.342
Bupivacaine	0.631	0.602	1.162	1.215	1.398	1.413	1.483
2-Chloroprocaine	0.647	0.616	1.195	1.252	1.362	1.371	1.434
Cocaine	0.648	0.620	1.165	1.216	1.390	1.404	1.490
Dibucaine	0.596	0.569	1.179	1.231	1.403	1.417	1.527
Dyclonine	0.620	0.593	1.196	1.247	1.372	1.386	1.466
Lidocaine	0.684	0.649	1.153	1.212	1.350	1.361	1.433
Phenytoin	0.715	0.682	1.123	1.174	1.334	1.343	1.491
Prilocaine	0.678	0.647	1.184	1.236	1.350	1.361	1.438
Procaine	0.654	0.622	1.208	1.268	1.350	1.359	1.434
(R)-Propanolol	0.660	0.627	1.166	1.224	1.368	1.382	1.446
(S)-Propanolol	0.654	0.626	1.178	1.228	1.358	1.371	1.457
Tetracaine	0.626	0.596	1.216	1.273	1.365	1.379	1.451

a Molecular globularity.
b Reference: calculations carried out with the GEPOL program.
c Molecular rugosity (Å–1).
d Fractal dimension of the solvent accessible surface.
e Fractal dimension of the solvent accessible surface averaged for non -buried atoms.

SplitsTree is a program for analyzing CA data [21]. Based on the method of split decomposition, it takes as input a distance matrix or a set of CA data and produces as output a graph that represents the relationships between the taxa. For ideal data this graph is a tree whereas less ideal data will give rise to a tree-like network, which can be interpreted as possible evidence for different and conflicting data. Furthermore, as split decomposition does not attempt to force data onto a tree, it can provide a good indication of how tree-like given data are. The splits graph for the 28 local anaesthetics of Table 1 (cf. Fig. 5) reveals no conflicting relationship between the anaesthetics. In particular compounds 1, 3, 4, 6, 11, 13–25 and 28 appear superimposed. The splits graph is in general agreement with both partial correlation diagrams, dendrograms and binary trees (Fig. 3-4). The main difference is the partial fusion of C_{b_2} classes (1,4,6,7,8,14,17,20,21,22,23) and (3,11,12,13,15,16,18,19,24,25,28). However, the results (Fig. 5) should be taken with care, because the former class includes four compounds with the constant <11111> vector (anaesthetics 4, 6, 20 and 23), for which the null standard deviation causes a Pearson correlation coefficient of r = 1 with any local anaesthetic, which is an artifact.

A principal component analysis (PCA) has been carried out for the local anaesthetics. The importance of PCA factors F1–5 for {i1,i2,i3,i4,i5} shows that the use of only the first factor F1 explains 35% of the variance (65% error); the combined use of the first two factors F1–2 explains 61% of the variance (39% error); the use of the first three factors F1–3 explains 78% of the variance (22% error). The PCA factor loadings are calculated.

Table 5. Predictions for topical anaesthetics and ice both not included in the models.

Anaesthetic	Ingredients	Prediction			Experiment	
		Onset	Potency	Duration	Relative potencya	Rel. pot. afterb
Betacaine-LA	lidocaine: prilocaine: dibucaine	rapid	moderate	moderate	1.0	1.0
ELA-max	4% lidocaine	Rapid	moderate	moderate	1.5	1.5
ELA-max 5	5% lidocaine	rapid	moderate	moderate	–	–
EMLA cream	2.5% lidocaine: 2.5% prilocaine	rapid	moderate	moderate	1.4	1.5
Tetracaine gel	4% tetracaine gel	slow	high	long	1.2	1.1
Amethocaine	4% tetracaine	slow	high	long	–	–
Topicaine	4% lidocaine	rapid	moderate	moderate	–	–
S-caine	2.5% lidocaine: 2.5% tetracaine	moderate	moderate-high	moderate-long	–	–
Hurricaine	20% benzocaine	rapid	–	short	–	–
Cetacaine	14% benzocaine: 2% tetracaine: 2% butamben	rapid	–	long	–	–
Lidocaine	2%, 4% lidocaine	moderate	moderate	moderate	–	–
Tetracaine	2% tetracaine	slow	high	long	–	–
Cocaine	cocaine	moderate	–	long	–	–
Icec		moderate	low	short	<1.4	<1.5

a From mean pain scorafter application of topical anaesthetics for 60 minutes.
b From mean pain scores 30 minutes after the 60-minute application period of anaesthetics.
c From mean pain scores after application of topical anaesthetics.

The PCA F1–5 profile for the vector property shows that for F1 and F5 variable i5 has the greatest weight in the profile; however, F1 cannot be reduced to three variables {i2,i3,i5} without a 14% error. For F2 variable i1 has the greatest weight; notwithstanding, F2 cannot be reduced to three variables {i1,i3,i4} without a 4% error. For F3 variable i2 has the greatest weight; nevertheless, F3 cannot be reduced to three variables {i2,i3,i4} without a 2% error. For F4 variable i4 has the greatest weight; however, F4 cannot be reduced to three variables {i1,i4,i5} without a 4% error. F1–2–3–4–5 can be considered as linear combinations of {i2,i3,i5}, {i1,i3,i4}, {i2,i3,i4}, {i1,i4,i5} and {i1,i3,i5} with 14%, 4%, 2%, 4% and 13% errors, respectively. In the F2– F1 plot, those local anaesthetics analogues of procaine with the same vector property appear superimposed. In particular anaesthetic 27 (class 4) also comes out placed over compounds 14 and 17 (class 1).

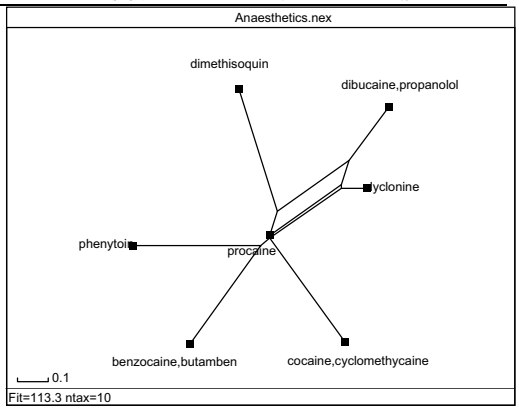


Figure 5. Splits graph for the local anaesthetics. Taxa 1, 3, 4, 6, 11, 13–19, 21–25 and 28 are hidden.

Table 6. Periodic properties for local anaesthetics (procaine analogues). Ice is included for comparison.

g000	g010	*g100*	g101	g110	g111
ice				diperodon, pramoxine, mexiletine	cocaine, cyclomethycaine
	dibucaine, propanolol		benzocaine, butamben	dyclonine	hexylcaine, piperocaine
	dimethisoquin			bupivacaine, etidocaine, lidocaine, mepivacaine, prilocaine, tocainide, S-ropivacaine	benoxinate, proparacaine, propoxycaine
		phenytoin			butacaine, 2-chloroprocaine, procaine, tetracaine

Five classes of anaesthetics are clearly distinguished: class 1 with 11 units ($0 \approx F1 < F2$, cf. Fig. 6 top), class 2 (11 units, $F1 > F2$, right), class 3 (2 units, $F1 << F2 \approx 0$, left), class 4 (1 unit, $-1 \approx F1 < F2 \approx 0$, middle) and class 5 (3 units, $F1 >> F2$, bottom). The classification is in agreement with both partial correlation diagrams, dendrograms, binary trees and splits graph (Fig. 3–5).

The recommended format for the periodic table (PT) of the local anaesthetics (cf. Table 6) shows that they are classified first by i5, then by i4, i3, i2 and, finally, by i1. Periods of five units are assumed. Group g010 stands for <i1,i2,i3> = <010>, viz. <01001> (dibucaine, propanolol), and <01010> (dimethisoquin), etc. The local anaesthetics in the same column appear close in both partial correlation diagrams, dendrograms, radial trees, splits graph and PCA (Fig. 3–6). The variation of vector property P = <i1,i2,i3,i4,i5> as a function of structural parameters {i1,i2,i3,i4,i5} shows that the lines for the structural parameters i4 and i5 appear superposed, what agrees with a PT of properties with vertical groups defined by {i1,i2,i3} and horizontal periods described by {i4,i5}. The variation of P = <i1,i2,i3,i4,i5>, as a function of the number of the group in PT, reveals that the minima correspond to <i1,i2,i3> = <010> (group g010). Corresponding function P(i1,i2,i3,i4,i5) reveals a series of waves clearly limited by maxima or minima, which suggest a periodic behaviour. For <i1,i2,i3,i4,i5> two minima are clearly shown. The distance between each pair of consecutive minima is five, which coincides with the local anaesthetic sets belonging to the same group in PT and in the successive periods. The minima occupy analogous positions in the curve and are in phase. The representative points in phase should correspond to the elements of the same group in PT. For the minima there is coherence between both representations; however, the consistency is not general. The comparison of the waves shows two differences: (1) periods 1–2 show some sawtooth-like structures with marked discontinuities in P(i1,i2,i3,i4,i5); (2) periods 3–4 are also sawtooth-like although much less marked. The most characteristic points of the plot are the minima, which correspond to group g010. The <i1,i2,i3,i4,i5> values are not repeated as the periodic law (PL) states but decrease regularly. An empirical function P(i1,i2,i3,i4,i5) reproduces the different <i1,i2,i3,i4,i5> values. A minimum of P(p) has meaning only if it is compared with the former P(p–1) and later P(p+1) points, needing to fulfil:

$$P_{\min}(p) < P(p-1)$$
$$P_{\min}(p) < P(p+1) \qquad (8)$$

Order relations (8) should repeat at determined intervals equal to the period size and are equivalent to:

$$P_{\min}(p) - P(p-1) < 0$$
$$P(p+1) - P_{\min}(p) > 0 \qquad (9)$$

As relations (9) are valid only for minima more general others are desired for all the values of p. The differences D(p) = P(p+1) – P(p) are calculated assigning each of their values to anaesthetic p:

$$D(p) = P(p+1) - P(p) \qquad (10)$$

Instead of D(p) the R(p) = P(p+1)/P(p) values can be taken assigning them to anaesthetic p. If PL were general, the elements in the same group in analogous positions in the different waves would satisfy:

$$D(p) > 0 \text{ or } D(p) < 0 \qquad (11)$$
$$R(p) > 1 \text{ or } R(p) < 1 \qquad (12$$

CONCLUSIONS

From the present results and discussion the folllowing conclusions can be drawn.

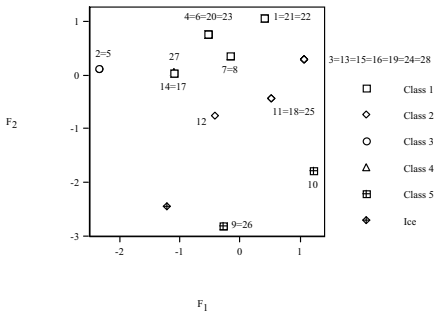
1. Several criteria, selected to reduce the analysis to a manageable quantity of structures from the large set of local anaesthetics, refer to the structural parameters related with the lipophilic portion, etc. Many algorithms for classification are based on information entropy. For sets of moderate size, an excessive number of results appear compatible with data, and the number suffers a combinatorial explosion. However, after the equipartition conjecture, one has a selection criterion between different variants resulting from classification between hierarchical trees. According to the conjecture, the best configuration of a flowsheet is the one in which the entropy production is most uniformly distributed. The method avoids the problem of other methods of continuum variables, because for the four compounds with constant <11111> vector, the null standard deviation always causes a Pearson correlation coefficient of r = 1. The lower level classification processes show lower entropy and may be more parsimonious.

2. Program MolClas, is a simple, reliable, efficient and fast procedure for molecular classification based on the equipartition conjecture of entropy production.

3. The good comparison of our classification results with other taken as good confirm the adequacy of the property vector selected for the molecular structures of the local anaesthetics. Information entropy and principal component analyses permit classifying the local anaesthetics and agree. The ester- and amide-type local anaesthetics are grouped in different classes. The agents of low potency and short duration are separated from those of high potency and long duration. The final classification is shown more precise and with lower bias. The classification model calculates in each case the contribution of signal and noise. MolClas has been written not only to analyze the equipartition conjecture of entropy production, but also to explore the world of molecular classification.

4. Topical anaesthetics remain a powerful, new advancement for minimizing pain during cutaneous procedures. While several new topical anaesthetic agents have been released that claim increased efficacy and faster onset, EMLA remains the most widely used topical anaesthetic given its proven efficacy and safety by several clinical trials. As the options for the practitioner continue to grow, the need for studies comparing onset of action, efficacy and safety continues to be of paramount importance. MolClas provides a way to classify the local anaesthtics for difficult cases that are hard to sort a priori, e.g., the relationship between procaine and ice. Both EMLA and ice decrease the discomfort associated with needle injection. Although EMLA performs better in pain control, ice has advantages in easy of use, fast action and is less expensive than EMLA. Both EMLA and ice are good topical anaesthetics, each with advantages and disadvantages in clinical use.

Figure. 6. PCA F2 vs. F1 plot for local anaesthetics. Ice is included for comparison.



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